The Institute of Paper Chemistry

Appleton, Wisconsin

Doctor's Dissertation

The Degradation of Ethyl 3,4,6-Tri-*O*-Methyl-β-D-*arabino*-Hexopyranosidulose in Aqueous Alkaline Hydrogen Peroxide Solution

Robert J. Niebauer

January, 1979

THE DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-*arabino*-HEXOPYRANOSIDULOSE IN AQUEOUS ALKALINE HYDROGEN PEROXIDE SOLUTION

A thesis submitted by

Robert J. Niebauer

B.S. 1973, Gannon College

M.S. 1975, Lawrence University

in partial fulfillment of the requirements
of The Institute of Paper Chemistry
for the degree of Doctor of Philosophy
from Lawrence University,
Appleton, Wisconsin

January, 1979

TABLE OF CONTENTS

SUMMARY

Ethyl 3,4,6-tri-O-methyl-β-D-arabino-hexopyranosidulose (ET3M-KG) was degraded at 25°C in 0.05N sodium hydroxide. Ethyl 4-deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (α,β-KG) was isolated from the reaction solution and identified as the initial degradation product by comparison of ^{1}H-NMR, ^{13}C-NMR, UV, and IR spectra with the known compound. The alkaline degradation of ET3M-KG was very rapid with a half-life of 1.2 minutes. α,β-KG acted as a reactive intermediate in this reaction reaching its maximum concentration just before all of the ET3M-KG had been consumed. Since α,β-KG was the first intermediate in the alkaline degradation of ET3M-KG, it was also degraded at 25°C in 0.05N sodium hydroxide. α,β-KG degraded much more slowly than ET3M-KG under these conditions. Analysis of methanol and ethanol liberated from ET3M-KG and α,β-KG showed that methoxy-containing products were being generated during the alkaline degradation reactions. A mechanism utilizing a series of base-catalyzed β-elimination reactions is presented to account for these observations. The mechanism was further supported by analysis of the reactions by ultraviolet spectroscopy.

ET3M-KG was also degraded in 0.1M hydrogen peroxide and 0.05N sodium hydroxide at 25°C. Care was taken to ensure minimum contamination from trace metals. α,β-KG was isolated and identified as the first formed intermediate in the reaction. Therefore, the hydrogen peroxide had no effect on the initial alkaline degradation of ET3M-KG. The alkaline peroxide reaction rate slowed early in the reaction, finally stopping completely after 15 minutes of reaction due to neutralization of the alkali by acidic reaction products. In the degradation of α,β-KG under identical conditions only 20% of the reactant and 60% of the original peroxide charge were consumed. Alcohol analyses showed that no methoxy- and/or ethoxy-containing products were generated.

A mechanism is postulated for the degradation of ET3M-KG involving Michael addition of hydroperoxide anion to the α,β-unsaturated carbonyl system in the intermediate α,β-KG. Presumably the resultant hydroperoxy ketone subsequently underwent an alkaline peroxide epoxidation reaction.

To investigate the implications of this work on the alkaline oxidative degradation mechanism of glycosides, ethyl 3,4,6-tri-O-methyl-β-D-glucopyranoside (ET3MG) was degraded at 60°C in 1.25N sodium hydroxide and 0.20M hydrogen peroxide. ET3MG degraded very slowly under these conditions and relatively large amounts of an organic peroxide were measured at an early reaction time. Intermediate organic peroxides have also been reported by other workers (1,2), who postulated that they were α-hydroxyhydroperoxides. α-Hydroxyhydroperoxides have been postulated to be in equilibrium with the parent carbonyl compound (3-5), but when attempts were made to deliberately form an α-hydroxyhydroperoxide from ET3M-KG, only insignificant amounts of organic peroxides were detected. This suggests that although the keto-glycoside, ET3M-KG, may exist in equilibrium with the α-hydroxyhydroperoxide, the equilibrium favors the keto-glycoside. Since relatively large amounts of organic peroxide were measured at early reaction times in the alkaline peroxide degradation of ET3M-KG, it can be postulated that the α-hydroxyhydroperoxide is the first intermediate formed in the alkaline oxidative degradation of glycosides. This postulation has been made previously (1,6,7) by workers studying the alkaline oxidation of glycosides.

INTRODUCTION

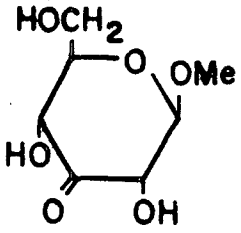
PERSPECTIVE

Alkaline peroxide has long been recognized as an effective bleaching agent which is primarily used for mechanical pulps. A German patent in 1905 is the earliest reference to its use for this purpose (8). Since that time much work has been carried out to optimize this delignification process (9-11). However, little attention has been paid to the chemistry involved in the oxidative degradation mechanism of carbohydrates in alkaline peroxide solutions.

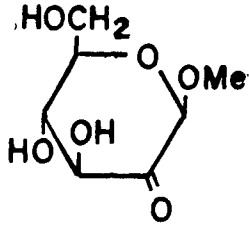
On a different front, much time, energy, and capital has been invested in the development of alkaline oxygen pulping and bleaching processes. The primary reason for this interest is that alkaline oxygen systems can potentially diminish pollution of air and water. The foremost problem associated with the alkaline oxygen treatment of wood and pulp is that severe carbohydrate degradation accompanies the delignification. The predominant form of cellulose degradation in these systems is a decrease in the degree of polymerization caused by cleavage of the cellulose chain.

Keto-glycosides have been postulated as early intermediates in both the alkaline peroxide and alkaline oxygen degradation of carbohydrates (1,6,7,12-19). In spite of the frequent proposal of these compounds as hypothetical intermediates, little is known about their chemistry under alkaline oxidative conditions.

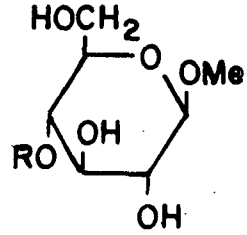
Theander (20) showed that methyl β-D-*ribo*-hexopyranosid-3-ulose (I) and methyl β-D-*arabino*-hexopyranosidulose (II) are extremely reactive in alkaline solutions. More recently, Ericsson, *et al.* (12) have studied the alkaline oxygen degradation of I and methyl β-D-glucopyranoside (III) and postulated that I was an intermediate in the alkaline oxidation of III. The same products



I: methyl β-D-ribo-hexopyranosid-3-ulose



II: methyl β-D-arabino-hexopyranosidulose



III: methyl β-D-glucopyranoside. R= hydrogen

IV: methyl 4-O-methyl-β-D-glucopyranoside. R= methyl

were formed whether oxygen or hydrogen peroxide was used as the oxidant in the alkaline oxidations (13). This finding was supported by the results obtained by other workers (6,14), who had shown that hydrogen peroxide was the reactive intermediate in the alkaline oxygen degradation of carbohydrates. Since the same oxidizing agent was present in both systems and identical products were found, the reaction mechanism in alkaline oxygen and in alkaline peroxide is believed to go through the same intermediates.

OXIDATION SITES IN ALKALINE OXIDATIVE DEGRADATIONS

Haskins and Hogsed (15), as a result of their work with the alkaline peroxide degradation of cellulose, concluded that oxidation at any of the three available hydroxyl groups on the anhydroglucose units of cellulose would

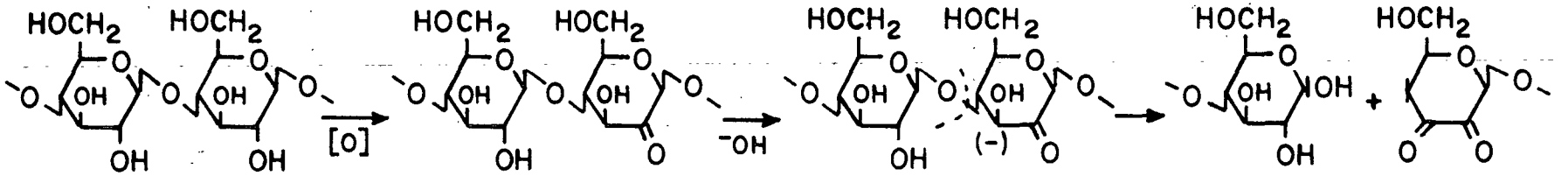
produce a product that could undergo chain cleavage reactions in basic solution (Fig. 1). The chain cleavage reactions were assumed to proceed by a β-alkoxy elimination mechanism, first proposed by Isbell (16).

Lewin and Ettinger (17) also studied the alkaline peroxide degradation of cellulose. They concluded that the secondary hydroxyl groups contribute more to the oxidation of cellulose than the primary hydroxyl at C-6. Samuelson and Thede (18), using hydrocellulose, have also suggested that, under alkaline oxygen conditions, oxidation occurs primarily at the secondary hydroxyl groups of the anhydroglucose units.

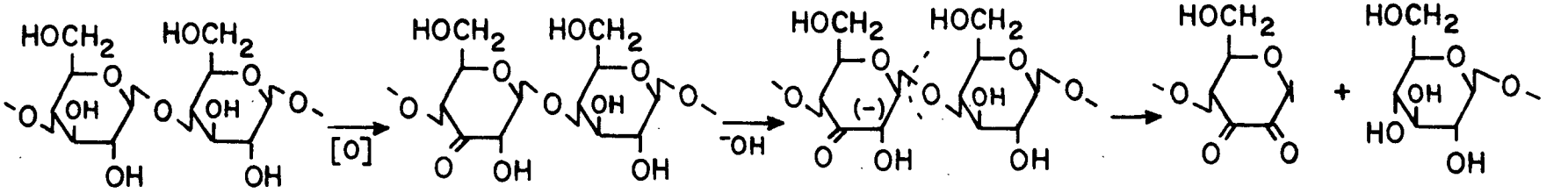
Due to the complexity of the mechanisms involved, cellulose model compounds have been studied to help elucidate the oxidative degradation mechanisms of cellulose under alkaline peroxide and alkaline oxygen conditions. Such models are usually well-characterized derivatives of D-glucose.

McCloskey (7), working with O-methylated derivatives of methyl β-D-glucopyranoside, concluded that the C-6 hydroxyl group has little influence on the degradation of methyl β-D-glucopyranoside. Other workers, using cellulose model compounds in alkaline oxidative systems, agree that oxidation at C-6 is of little importance (1,19,21).

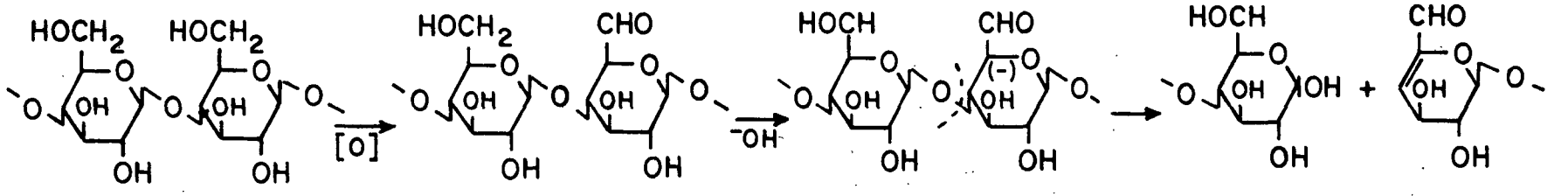
Work with alkyl 4-O-methyl glucosides suggests that C-4 elimination is slightly preferred over C-1 elimination. Based on the alkaline oxygen degradation of ethyl glucosides, Kolmodin (19), concluded that β-alkoxy elimination of the C-4 methoxyl groups was favored over elimination of the alkoxyl group from C-1. He further proposed that this elimination proceeded through a keto-glycoside intermediate in which the carbonyl was located at C-2. Weaver (1) presented a similar conclusion as a result of a study of the alkaline peroxide degradation of methyl 4-O-methyl-β-D-glucopyranoside (IV). Weaver credited



(a) Oxidation at C-2



(b) Oxidation at C-3



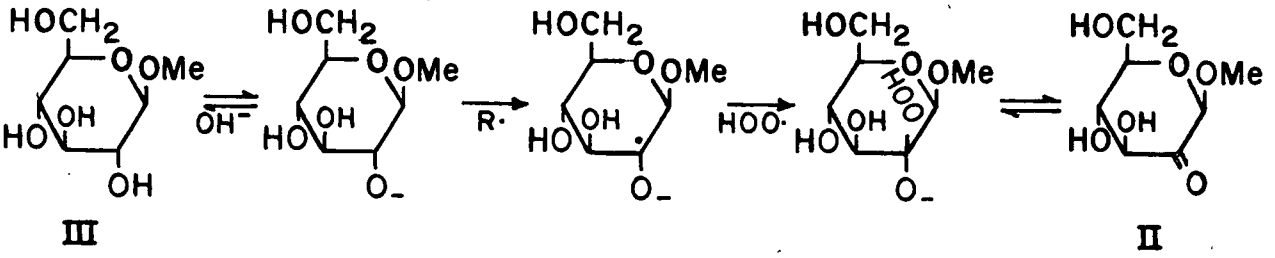
(c) Oxidation at C-6

Figure 1. Sites of Oxidation in the Cellulosic Chain (15)

the preferential formation of a C-2 carbonyl to the fact that the C-2 hydroxyl group in IV is the most acidic hydroxyl group in the molecule.

FORMATION OF THE KETO-GLYCOSIDES

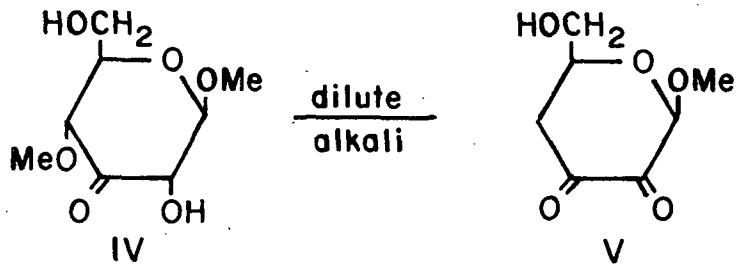
Although it is generally agreed that the alkaline oxidative degradation of carbohydrates proceeds through a keto-glycosidic intermediate, the mechanism by which these intermediates are formed remains highly speculative. It has been suggested that the carbonyl group of the keto-glycoside is formed from an organic peroxide precursor (1,2,6,7,22). Weaver's experimental data supported the interpretation that this precursor was an α-hydroxyhydroperoxide (1). This hydroperoxide was presumably formed by reaction of the glycoside with radicals formed from hydrogen peroxide decomposition. The mechanism that was postulated follows:



The C-2 hydroxyl group is the most acidic of the hydroxyls in III. Therefore, it is the preferred site of ionization. It should be noted that although Weaver (1) reported good evidence for the existence of an α-hydroxyhydroperoxide in his work with the alkaline peroxide degradation of III, this mechanism is highly speculative.

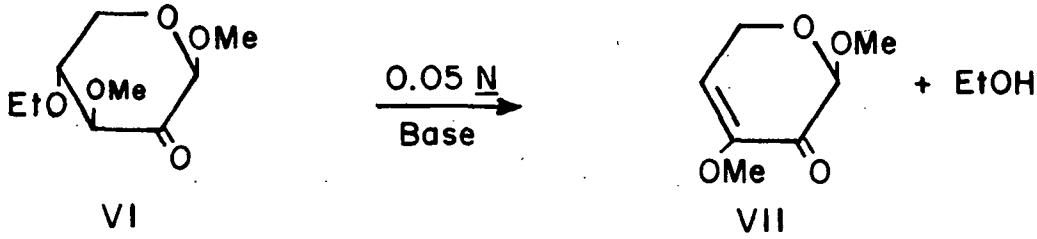
REACTIONS OF KETO-GLYCOSIDES

The most important reaction of keto-glycosides in alkaline solution is β-alkoxy elimination. Theander (20,21,23) has published several articles on the alkaline degradation of methyl β-D-ribo-hexopyranosid-3-ulose (I) and methyl β-D-arabino-hexopyranosidulose (II). In 0.018N sodium hydroxide at 25°C these keto-glycosides have half-lives of less than 5 minutes (20). The 4-O-methyl derivative of I is five times as reactive as the parent compound and the predominant product of this reaction was identified as methyl 4-deoxy-β-D-glycero-hexopyranosid-2,3-diulose (V), formed by β-elimination of the C-4 methoxyl group (23). This implies that even though the carbonyl is initially



at the 3 position, the majority of the reaction goes through a 2-keto intermediate, in which the site of elimination would be in the β-position to the carbonyl.

Kenne and Svensson (24) also studied alkaline degradation of etherified keto-glycosides. Working with methyl 4-O-ethyl-3-O-methyl-β-D-threo-pentopyranosidulose (VI) in a solution of sodium methoxide in methanol-dichloromethane, they obtained methyl 4-deoxy-3-O-methyl-β-D-pent-3-enopyranosidulose (VII) in an 84% yield after a short reaction time. In that time there was

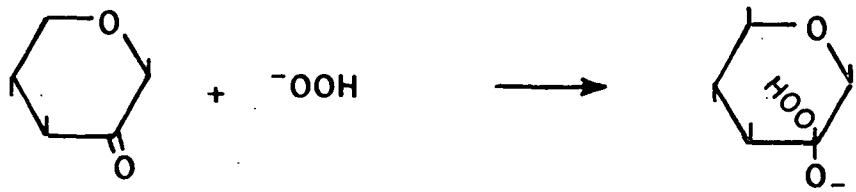


VI VII

0.97 mole of ethanol released and 0.13 mole of methanol. This indicates complete elimination of the C-4 ethoxyl group and elimination of some methanol from either C-1 or C-3. Kenne and Svensson (24) did not speculate on a mechanism for the liberation of the methanol.

EFFECT OF HYDROGEN PEROXIDE ON REACTIONS OF KETO-GLYCOSIDES

The hydroperoxide anion formed in solutions of alkaline hydrogen peroxide can act as a nucleophile and add to the carbonyl carbon of a ketone (25-29). Therefore the reactions of keto-glycosides in alkaline peroxide solutions can

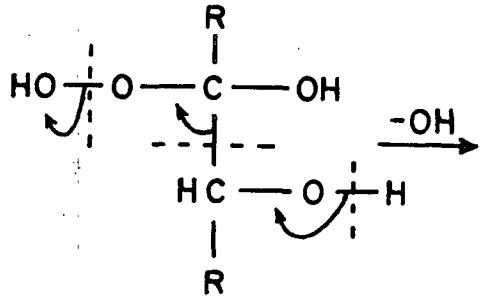


be attributed to either the keto form or the α-hydroxyhydroperoxide form. Reactions typical of α-hydroxyhydroperoxides are carbon-carbon bond cleavage and stepwise free radical or ionic degradation reactions (1,26,29), while reactions typical of keto-glycosides have been discussed in the previous section.

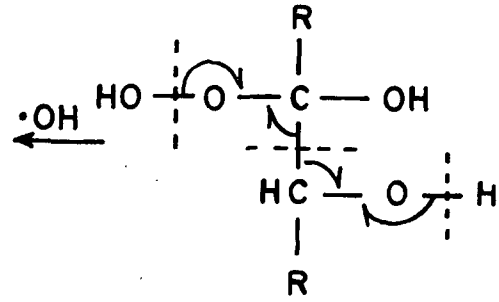
Weaver (1) has reported evidence for the existence of an α-hydroxyhydroperoxide intermediate in the alkaline peroxide degradation of methyl β-D-

glucopyranoside (III). He postulated that nucleophilic addition of the hydroperoxide anion to the carbonyl carbon of the keto-glycoside stabilized the keto-glycoside with respect to alkaline β-alkoxy elimination reactions (1). Due to this stability, it has been suggested (1,22,30) that α-hydroxyhydroperoxides may be important intermediates in the alkaline oxidation of methyl glycosides that lead to acidic products which retain their methoxy substituents.

Isbell, et al. (26-29) have studied the alkaline peroxide degradation of various carbonyl compounds. The hydroperoxide adduct can reportedly undergo ionic and free radical degradation reactions leading to one and two-carbon fragments (26):

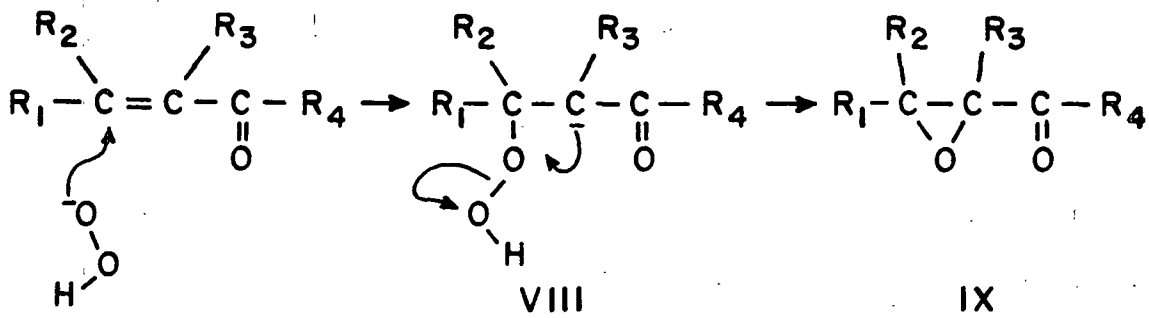


ionic mechanism



radical mechanism

The hydroperoxide anion may also act as a nucleophile and attack the conjugated system of an α,β-unsaturated ketone (31-33). The resulting β-hydroperoxy ketone (VIII) can subsequently undergo base-catalyzed epoxidation to yield an α,β-epoxy ketone [IX, (32)].



VIII IX

STATEMENT OF PURPOSE

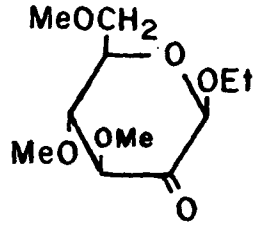
Very little is known about the chemistry of keto-glycosides in alkaline peroxide solutions. Yet, these extremely alkali-labile compounds have frequently been proposed as intermediates in carbohydrate degradation mechanisms in both alkaline peroxide (1,12,13) and alkaline oxygen (2,6,7,12,13,18,20-22,30) systems.

Therefore, it was the purpose of this work to: (1) select a keto-glycoside model compound; (2) synthesize and purify the keto-glycoside; (3) thoroughly evaluate the behavior of the keto-glycoside in alkaline solution and speculate on its alkaline degradation reaction mechanism; (4) investigate the effect that hydrogen peroxide has on the alkaline degradation; and (5) study the alkaline peroxide degradation of the parent glycoside from which the selected keto-glycoside was derived and the importance of the keto-glycoside as a possible intermediate in its oxidative degradation.

RESULTS AND DISCUSSION

SELECTION OF KETO-GLYCOSIDE SUBSTRATE

Ethyl 3,4,6-tri-O-methyl-β-D-arabino-hexopyranosidulose (ET3M-KG) was selected as the keto-glycoside to be studied in this work for several reasons.



ET3M-KG

Etherification of all of the available hydroxyl groups in this model simplified the number of possible initial degradation reactions. Since the carbonyl group cannot migrate to any other position on the ring, initial β-elimination reactions are directed toward sites in positions beta to the carbonyl.

The model was designed with an ethyl group at C-1 and methyl groups at the remaining hydroxyl positions so that elimination at C-1 could be distinguished from elimination at any other position on the ring. In this way insights into the mechanism could be obtained by the rate and amount of alcohols liberated during the reaction.

Although much work has been performed on the alkaline degradation of etherified keto-glycosides (24), mechanisms, other than for the initial step of the degradation, have not been proposed.

SYNTHESIS OF MODEL COMPOUNDS

Degradation reactions were performed on model compounds ethyl 3,4,6-tri-O-methyl-β-D-arabino-hexopyranosidulose (ET3M-KG), ethyl 4-deoxy-3,6-di-O-methyl-

β-D-glycero-hex-3-enopyranosidulose (α,β-KG) and ethyl 3,4,6-tri-O-methyl-β-D-glucopyranoside (ET3MG). Compound ET3MG was synthesized by a procedure described by Schroeder, et al. (34). Compounds ET3M-KG and α,β-KG are new compounds, and their characterization is presented in the Experimental section of this report. The synthetic route in the preparation of these model compounds is given in Reaction Scheme 1. The numbered compounds in this scheme are described in the Experimental section.

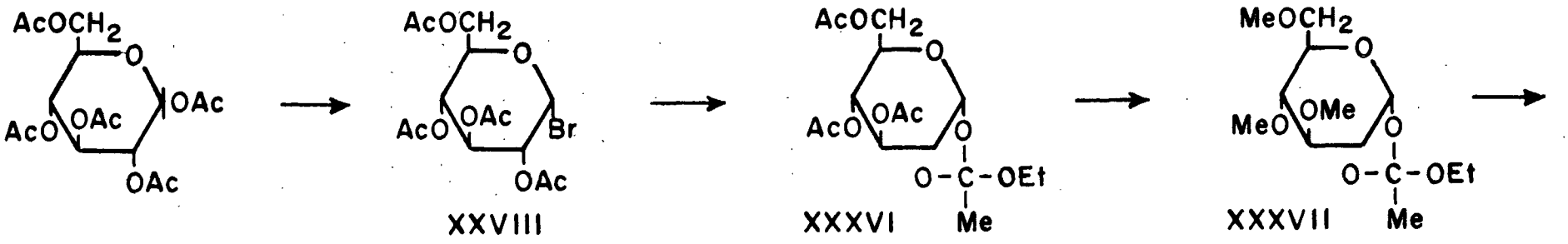
ALKALINE DEGRADATION REACTIONS

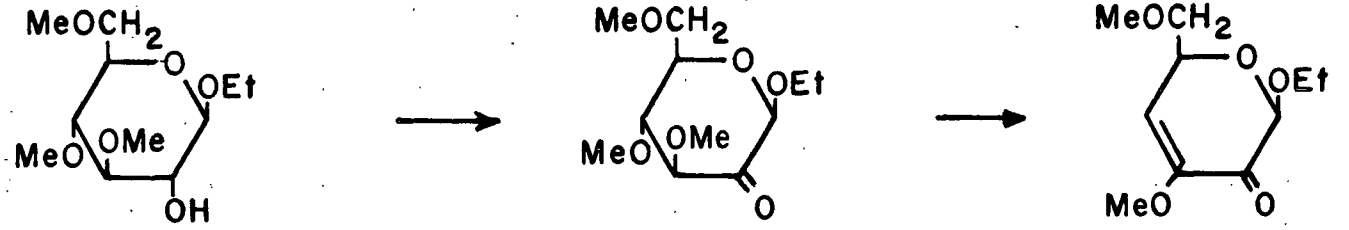
INTRODUCTION

To determine the effect that hydrogen peroxide has on the alkaline degradation of ethyl 3,4,6-tri-O-methyl-β-D-arabino-hexopyranosidulose (ET3M-KG), it was necessary to analyze thoroughly the degradation of the compound in alkali alone.

ET3M-KG (0.1M) was degraded at 25°C in 0.05N sodium hydroxide solution in a passivated glass reaction flask. These conditions were chosen on the basis of previous, related studies (12,21,23,24,35). The sodium hydroxide was purified to reduce extraneous metal ion contamination. No attempt was made to run the reaction under an inert atmosphere. Reaction samples were quenched immediately with sodium borohydride, which reduced the unreacted ET3M-KG to the epimers, ethyl 3,4,6-tri-O-methyl-β-D-mannopyranoside (ET3MM) and ethyl 3,4,6-tri-O-methyl-β-D-glucopyranoside (ET3MG). The glycosides were analyzed by quantitative gas-liquid chromatography (GLC) using cyclohexyl 3,4,6-tri-O-methyl-β-D-glucopyranoside (CT3MG) as an internal standard.

Figure 2 shows that the alkaline degradation of ET3M-KG was very rapid. As soon as the alkaline solution contacted the keto-glycoside, the solution





Reaction Scheme I

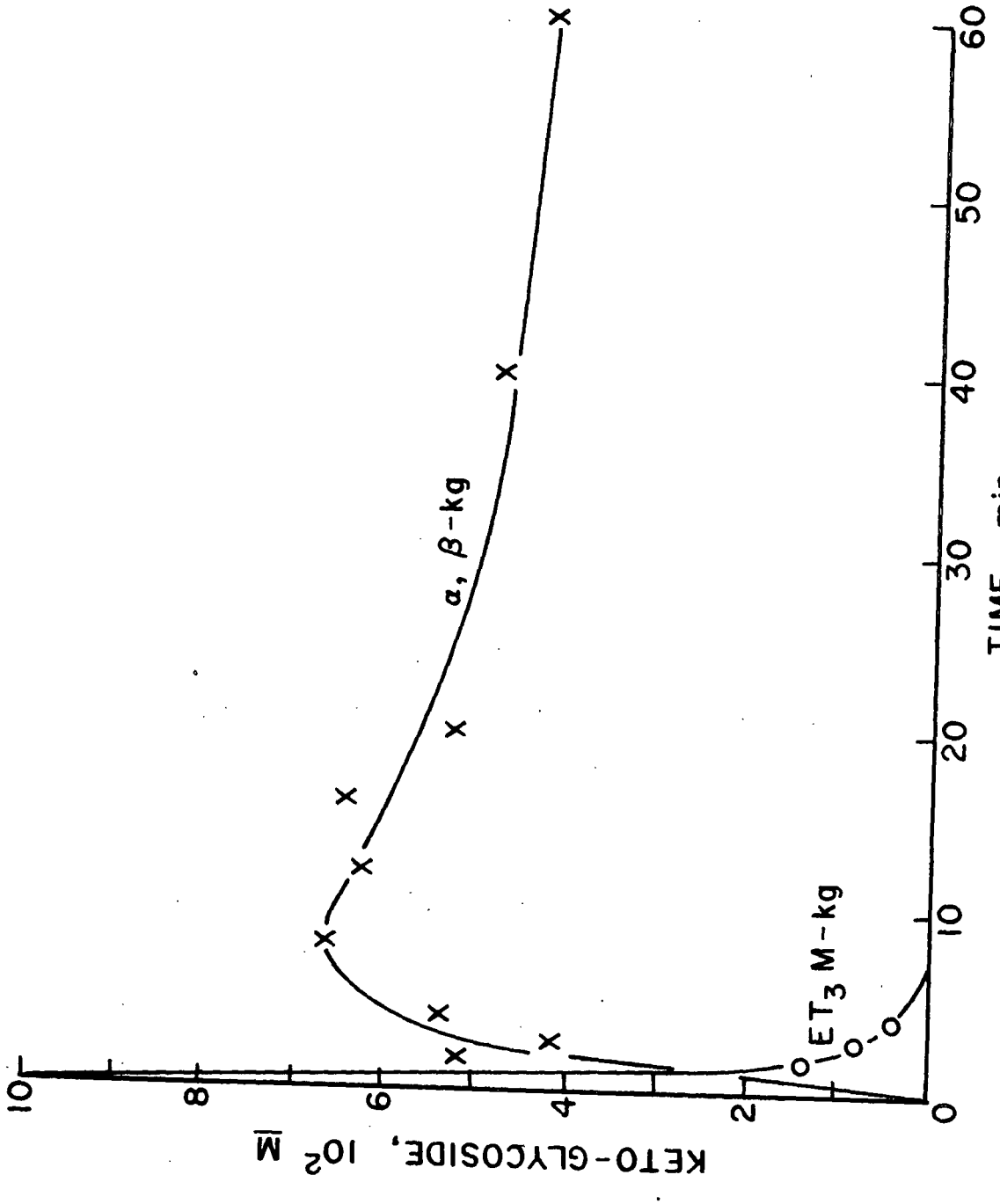
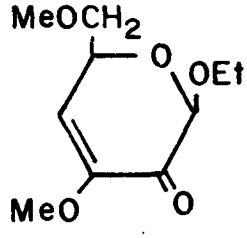


Figure 2. Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (ET3M-KG) and Formation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (α,β-KG) at 25°C in 0.05N NaOH

became yellow, indicating that chromophoric products were rapidly generated. During the degradation a neutral product, ethyl 4-deoxy-3,6-di-O-methyl-β-D glycero-hex-3-enopyranosidulose (α,β-KG) was formed. Figure 2 shows that α,β-KG behaved like a reactive intermediate in the alkaline system.



α,β-KG

Since α,β-KG is an important intermediate in the alkaline degradation of ET3M-KG, it was desirable to investigate its behavior in alkaline solution. Therefore α,β-KG was also degraded at 25°C in a 0.05N sodium hydroxide solution. The degradation was performed under conditions identical to those for degradation of ET3M-KG. Reaction samples were quenched with 4M acetic acid and immediately separated by preparative thin-layer chromatography (TLC). Unreacted α,β-KG was extracted from the developed chromatoplates, and its concentration was determined colorimetrically.

The alkaline degradation of α,β-KG is displayed in Fig. 3. The unsaturated keto-glycoside was much more stable in alkaline solution than ET3M-KG. However, as soon as the alkaline solution came into contact with the keto-glycoside it immediately turned yellow, similar to the ET3M-KG reaction.

ALKALI CONSUMPTION

Throughout the alkaline reactions of ET3M-KG and α,β-KG the pH was monitored by immersion of pH electrodes into the reaction solution.

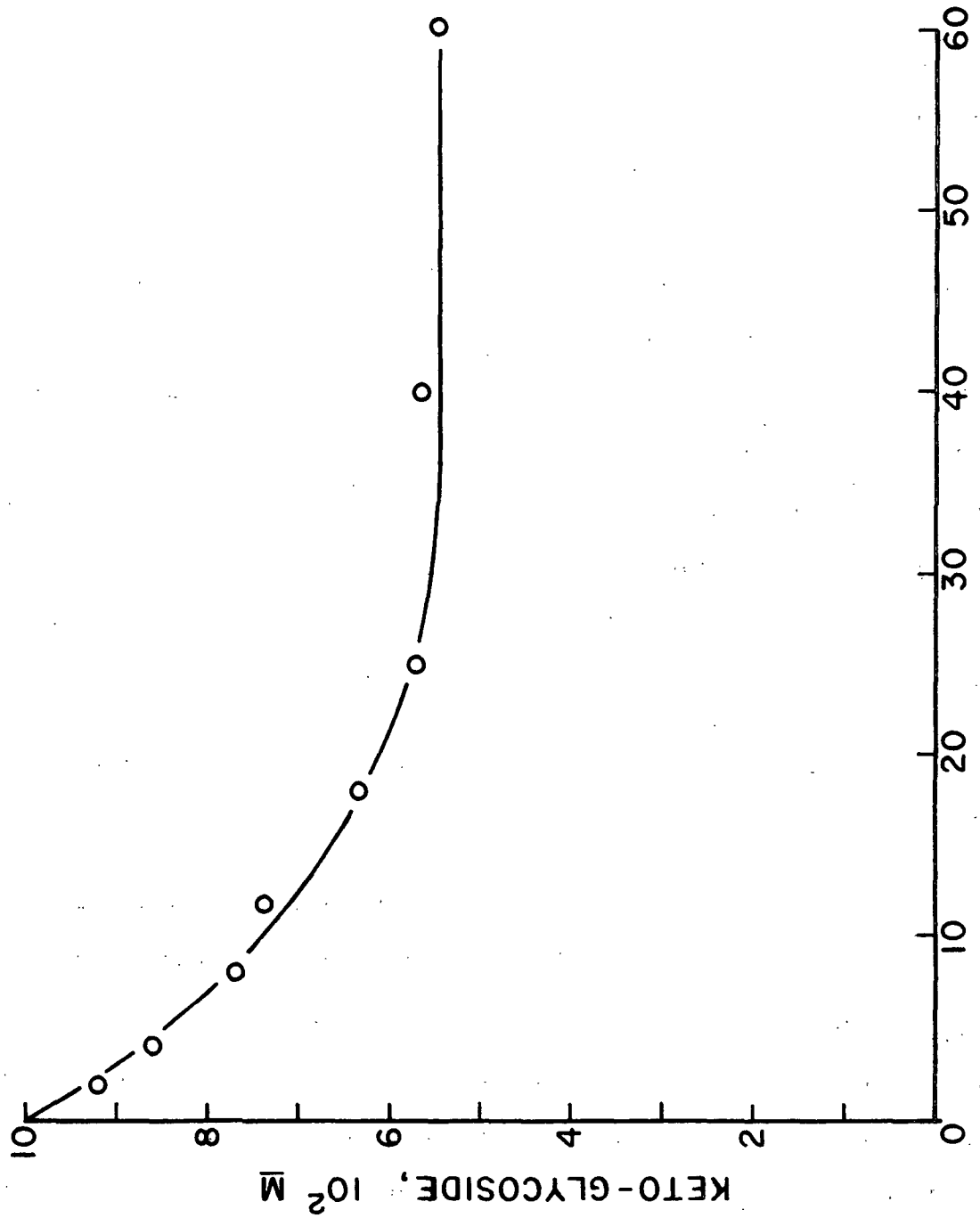


Figure 3. Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose in 0.05N NaOH at 25°C

Figure 4 shows that during the alkaline degradation of ET3M-KG the alkali concentration dropped from 0.05N to 0.004N. Figure 5 demonstrates that an almost identical drop occurred in the alkaline degradation of α,β-KG. The alkali was consumed by acidic products generated during the reaction.

PRODUCT ANALYSIS

Alcohols

The formation of methanol and ethanol in the alkaline degradation of ET3M-KG was studied as a function of time using quantitative GLC against n-propyl alcohol as the internal standard. Initially, methanol formation was very rapid, but subsequent liberation of methanol occurred at a much slower rate (Fig. 6). Consistent with the hypothesis that α,β-KG is the first intermediate in the reaction. The rapid initial formation of methanol corresponds to the rapid degradation of ET3M-KG (Fig. 7). The methanol formed after all of the ET3M-KG has degraded obviously must come from alkaline degradation products.

Ethanol was formed throughout the entire reaction (Fig. 6) and was roughly equivalent to the alkali consumed in the reaction (Fig. 4).

Figure 8 shows the formation of methanol and ethanol during the alkaline degradation of α,β-KG. The amount of ethanol liberated in this reaction was approximately the same as in the degradation of ET3M-KG under identical conditions. Likewise, after accounting for the equivalent of methanol released from ET3M-KG to form α,β-KG, the amount of methanol formed during α,β-KG degradation was approximately equivalent to the amount liberated during ET3M-KG degradation. Similar to the alkaline degradation of ET3M-KG, the formation of methanol in the alkaline degradation of α,β-KG was initially quite rapid and leveled off after 8 minutes of reaction time.

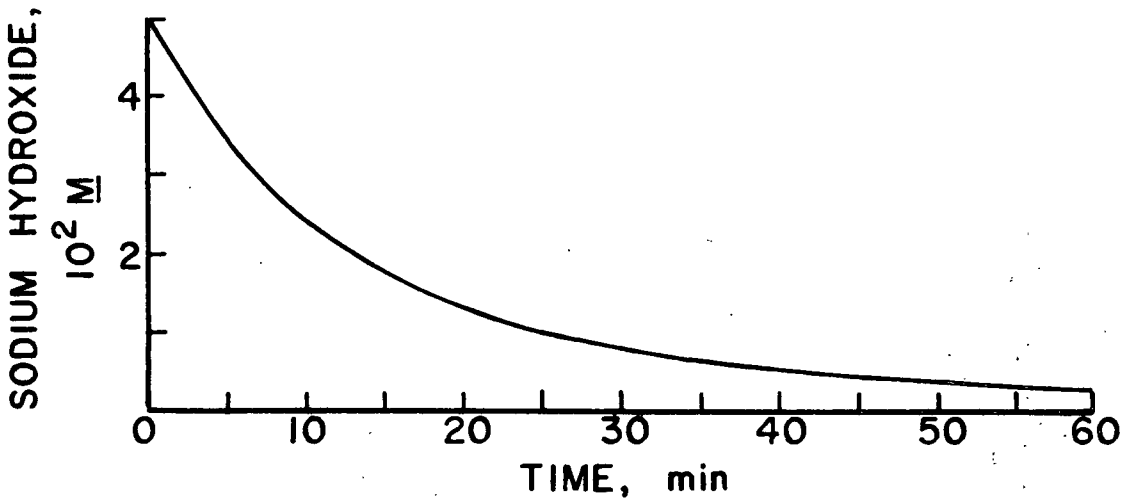


Figure 4. Change in Alkali Concentration During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH at 25°C

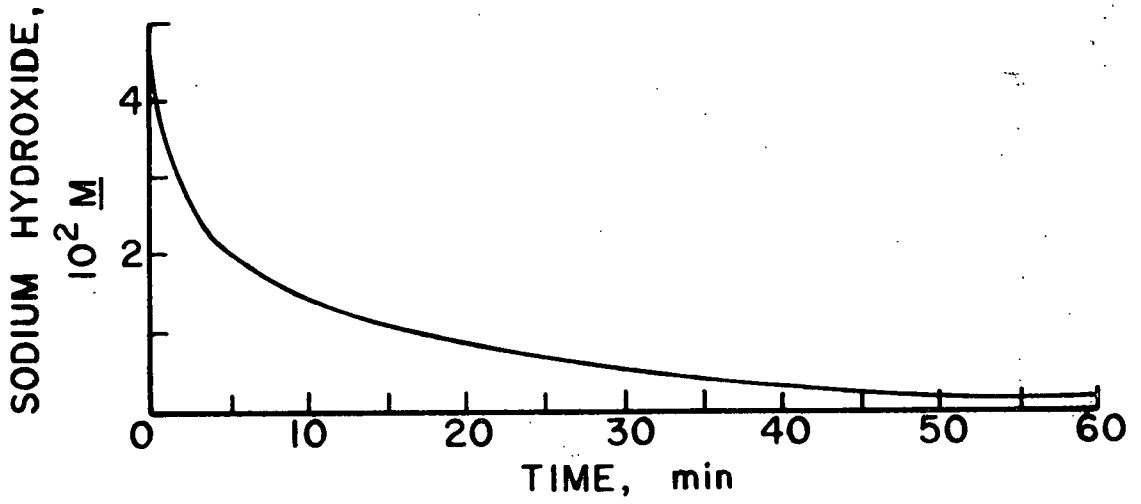


Figure 5. Change in Alkali Concentration During the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (0.10M) in 0.05N NaOH at 25°C

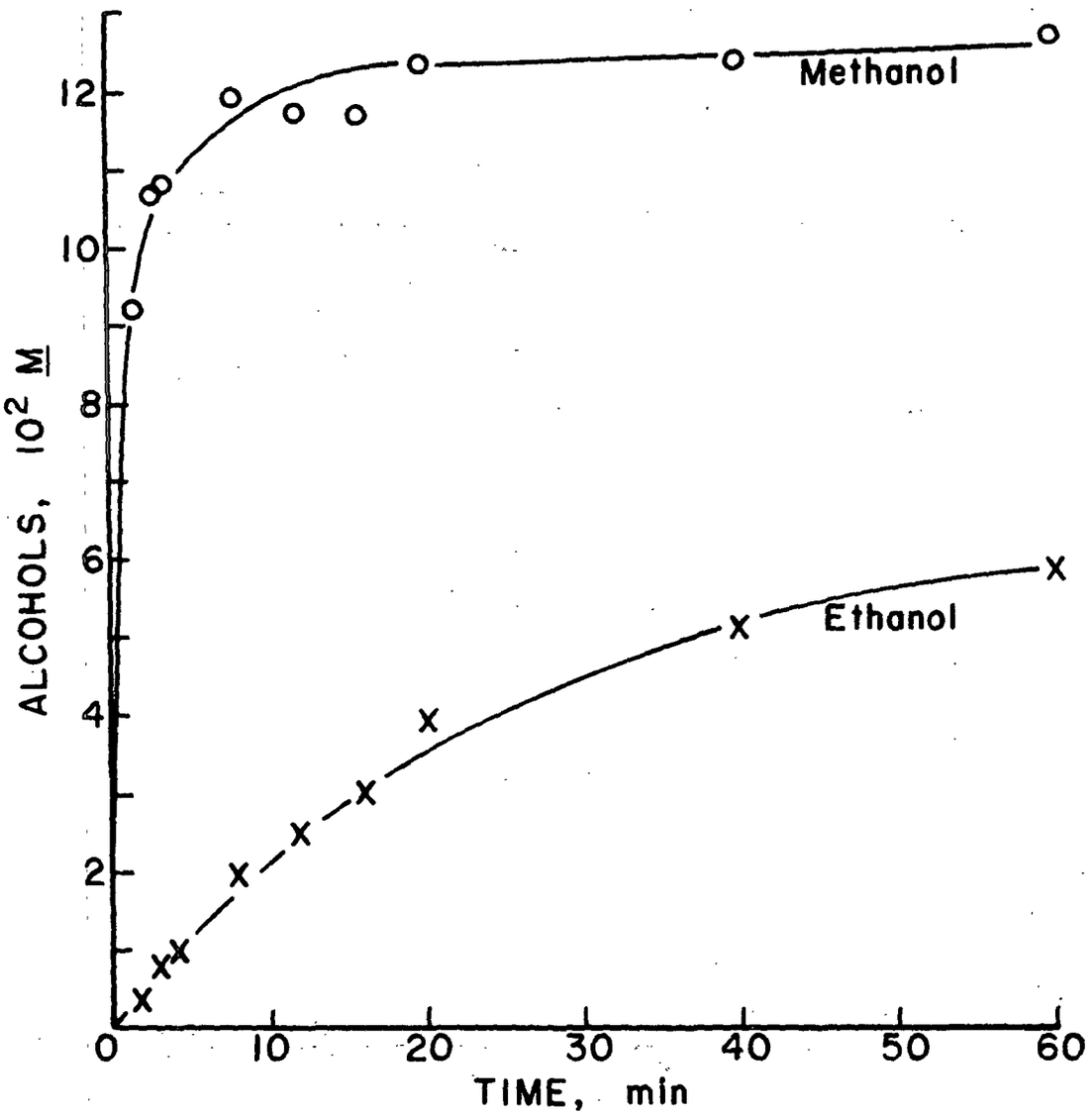


Figure 6. Formation of Methanol and Ethanol During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH at 25°C

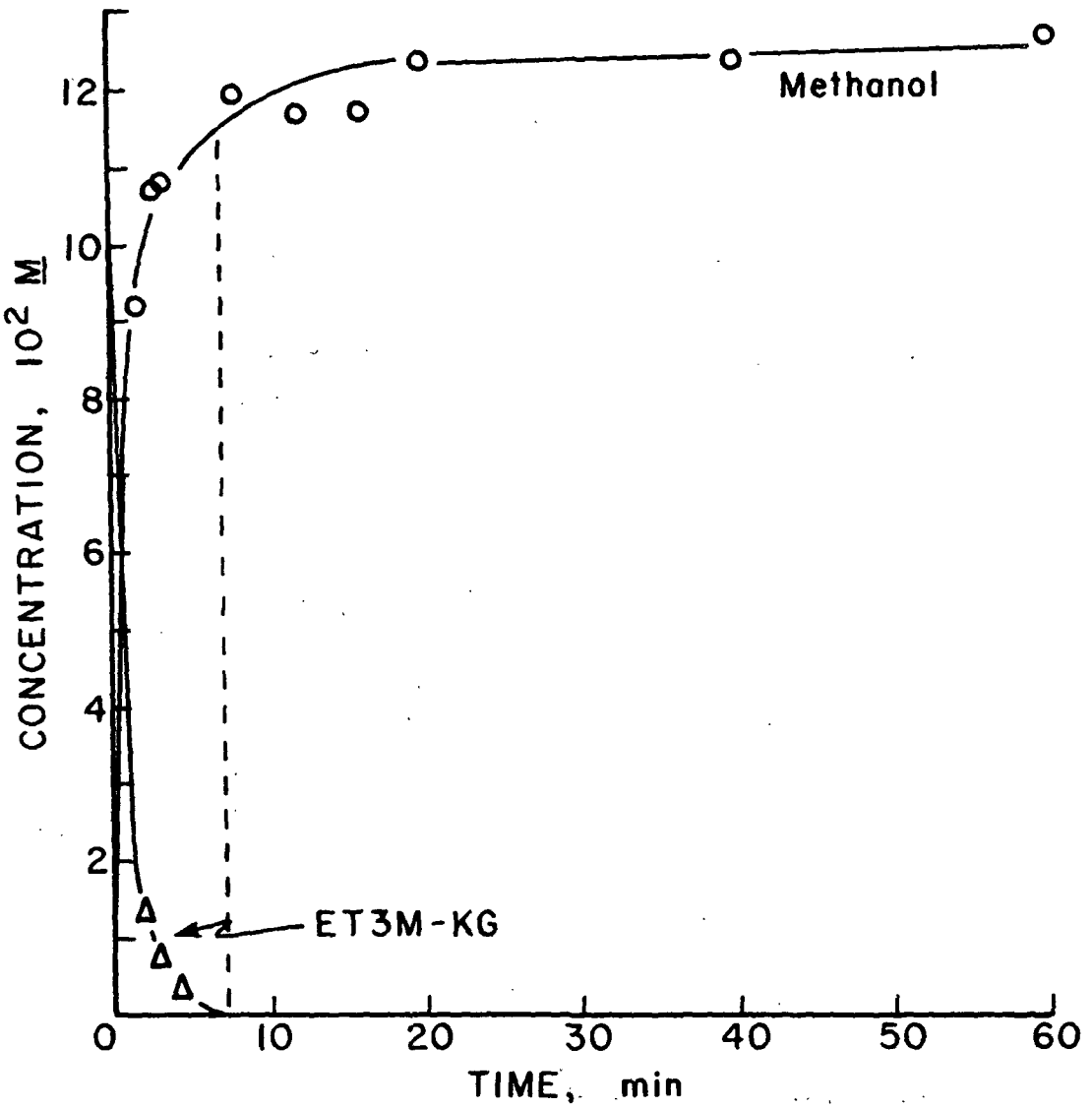


Figure 7. Formation of Methanol During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH at 25°C

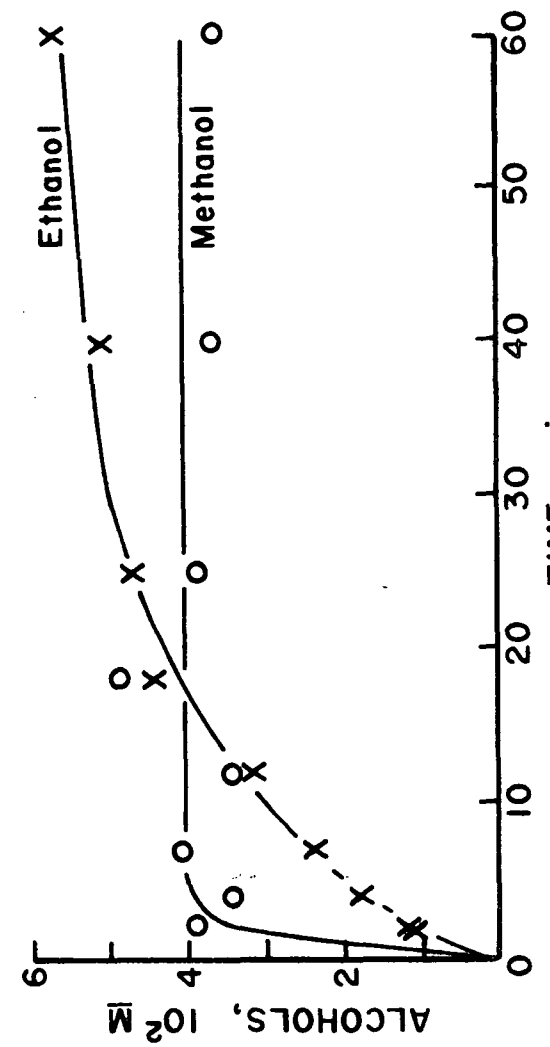


Figure 8. Formation of Methanol and Ethanol During the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (0.10M) in 0.05N NaOH at 25°C

Molar balances on the methoxy and ethoxy groups of the keto-glycosides during their alkaline degradation should give an insight to the mechanism that was operating. The alkoxy groups that could be accounted for (either as liberated alcohol or alkoxyl groups in α,β-KG and ET3M-KG) throughout the alkaline degradation of ET3M-KG, have been expressed in Table I and Fig. 9 as the molar percent of the substituents in the initial keto-glycoside. The methoxy balance was accomplished by summing the concentrations of liberated methanol, α,β-KG (2 equivalents of methoxy groups/equivalent α,β-KG) and ET3M-KG (3 equivalents of methoxy groups/equivalent of ET3M-KG); the ethoxy balance was made by summing the concentrations of liberated ethanol, α,β-KG and ET3M-KG. Each sum was then divided by the amount of alkoxy group in the initial keto-glycoside to arrive at the molar percent of alkoxy group of the initial keto-glycoside.

Figure 9 displays graphically how the molar percentages of alkoxy groups changed with time in the alkaline degradation of ET3M-KG. All of the ethoxy groups could be accounted for after one hour of reaction. The fact that it takes one hour to reach 100% of the theoretical amount of ethoxy groups can be explained by postulating that unanalyzed products which retained their ethoxy groups were formed, and that these products had limited stability in the alkaline solution.

The observation that the percentage methoxy groups accounted for continually decreased throughout the ET3M-KG degradation can be rationalized by postulating the formation of unanalyzed reaction products that retained one or more of their methoxy substituents.

Similar alkoxy balances were performed with the alkaline degradation of α,β-KG. Table II and Fig. 10 show that as the reaction proceeded the molar percentage of methoxy groups in α,β-KG accounted for decreased to approximately

TABLE I

MOLAR BALANCE ON ALKOXY SUBSTITUENTS FOR THE ET3M-KG ALKALINE DEGRADATION

Reaction Time, min	ET3M-KG x 10^2M	α,β-KG x 10^2M	MeOH x 10^2M	EtOH x 10^2M	Total MeO x 10^2M	Total EtO x 10^2M	Molar MeO, %	Molar EtO, %
0.0	0.100	--	--	--	0.300	0.100	100.0	100.0
1.0	0.041	0.032	0.092	0.005	0.279	0.078	93.0	78.0
2.0	0.017	0.036	0.102	0.008	0.225	0.061	75.0	61.0
4.0	0.003	0.054	0.107	0.011	0.224	0.068	74.7	68.0
8.0	0.000	0.067	0.113	0.018	0.247	0.085	82.3	85.0
12.0	0.000	0.062	0.116	0.024	0.240	0.086	80.0	86.0
16.0	0.000	0.058	0.119	0.030	0.235	0.088	78.3	88.0
20.0	0.000	0.055	0.121	0.035	0.231	0.090	77.0	90.0
40.0	0.000	0.046	0.125	0.051	0.217	0.097	72.3	97.0
60.0	0.000	0.044	0.126	0.058	0.214	0.102	71.3	102.0

TABLE II

MOLAR BALANCE ON ALKOXY SUBSTITUENTS FOR THE α,β-KG ALKALINE DEGRADATION

Reaction Time, min	α,β-KG x 10^2M	MeOH x 10^2M	EtOH x 10^2M	Total MeO x 10^2M	Total EtO x 10^2M	Molar MeO, %	Molar EtO, %
0.0	0.101	--	--	0.202	0.101	100.0	100.0
1.0	0.093	0.022	0.008	0.208	0.101	103.0	100.0
2.0	0.091	0.030	0.010	0.212	0.102	105.0	101.0
4.0	0.084	0.038	0.018	0.206	0.102	102.0	101.0
8.0	0.077	0.040	0.025	0.194	0.102	96.0	101.0
12.0	0.071	0.040	0.031	0.182	0.102	90.1	101.0
15.0	0.066	0.040	0.035	0.172	0.101	85.1	100.0
20.0	0.061	0.040	0.042	0.162	0.103	80.2	102.0
40.0	0.055	0.040	0.053	0.150	0.108	74.3	107.0
60.0	0.054	0.040	0.057	0.148	0.111	73.3	110.0

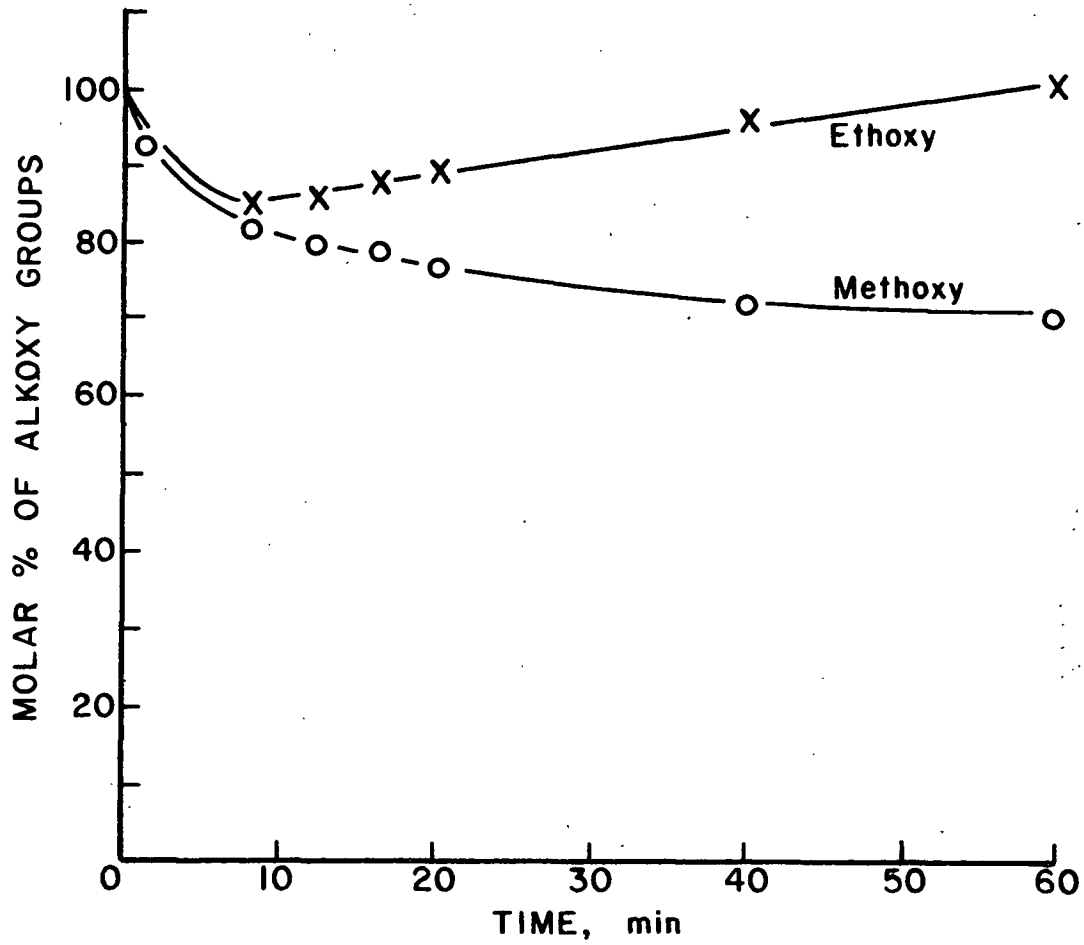


Figure 9. Percent of Alkoxy Groups Accounted for During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH at 25°C

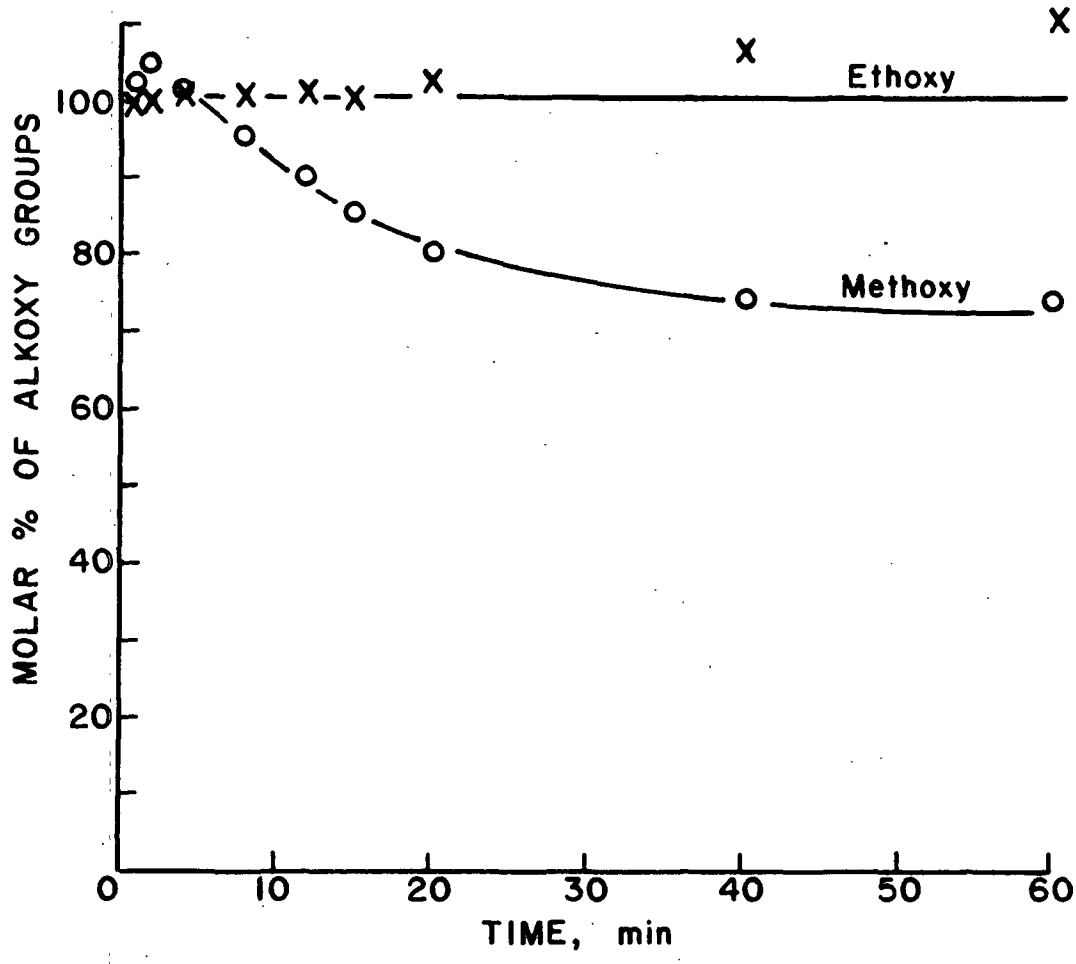


Figure 10. Percent of Alkoxy Groups Accounted for During the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hexopyranosidulose (0.10M) in 0.05N NaOH at 25°C

the same level that was observed in the alkaline degradation of ET3M-KG. The molar percent of ethoxy groups accounted for remained at ca. 100% throughout the entire reaction. These balances indicate that during this reaction a product(s) was formed that contained one or more methoxy groups but no ethoxy group.

Other Products

Ultraviolet (UV) Absorption Analysis

Because of the rapid rate of alkaline degradation of ET3M-KG, a technique utilizing UV spectrophotometry was used to analyze the reaction. With this technique, it was shown that ET3M-KG ($\lambda_{max}^{H_2O}$ 255 nm, ε ca. 10-20) underwent rapid alkaline degradation to form α,β-KG ($\lambda_{max}^{H_2O}$ 263 nm, ε 4,400). The strong absorption of the major product of the alkaline degradation reaction was utilized by initiating a reaction in a UV cuvette and following the increase in absorbance at 260 nm with time. The average half-life of several replicate reactions was 1.1 minutes. These reactions reached a constant maximum absorbance by 30 minutes, at which time a scan of the reaction solution from 200 nm to 400 nm showed a stronger absorption maximum at 317 nm. When the reaction solution was scanned from 220 nm to 400 nm, 6 hours after reaction initiation, another strong absorption maximum appeared at 284 nm (Fig. 11).

When α,β-KG was treated in the same manner it immediately formed a chromophore that absorbed at 317 nm. The average half-life of replicate reactions was 14 minutes.

Figure 11 was constructed by superimposing several rapid scans from 200 nm to 400 nm that were taken during an alkaline degradation of ET3M-KG. This figure shows that the chromophore which absorbed at 284 nm seemed to be formed at the expense of the chromophore absorbing at 317 nm.

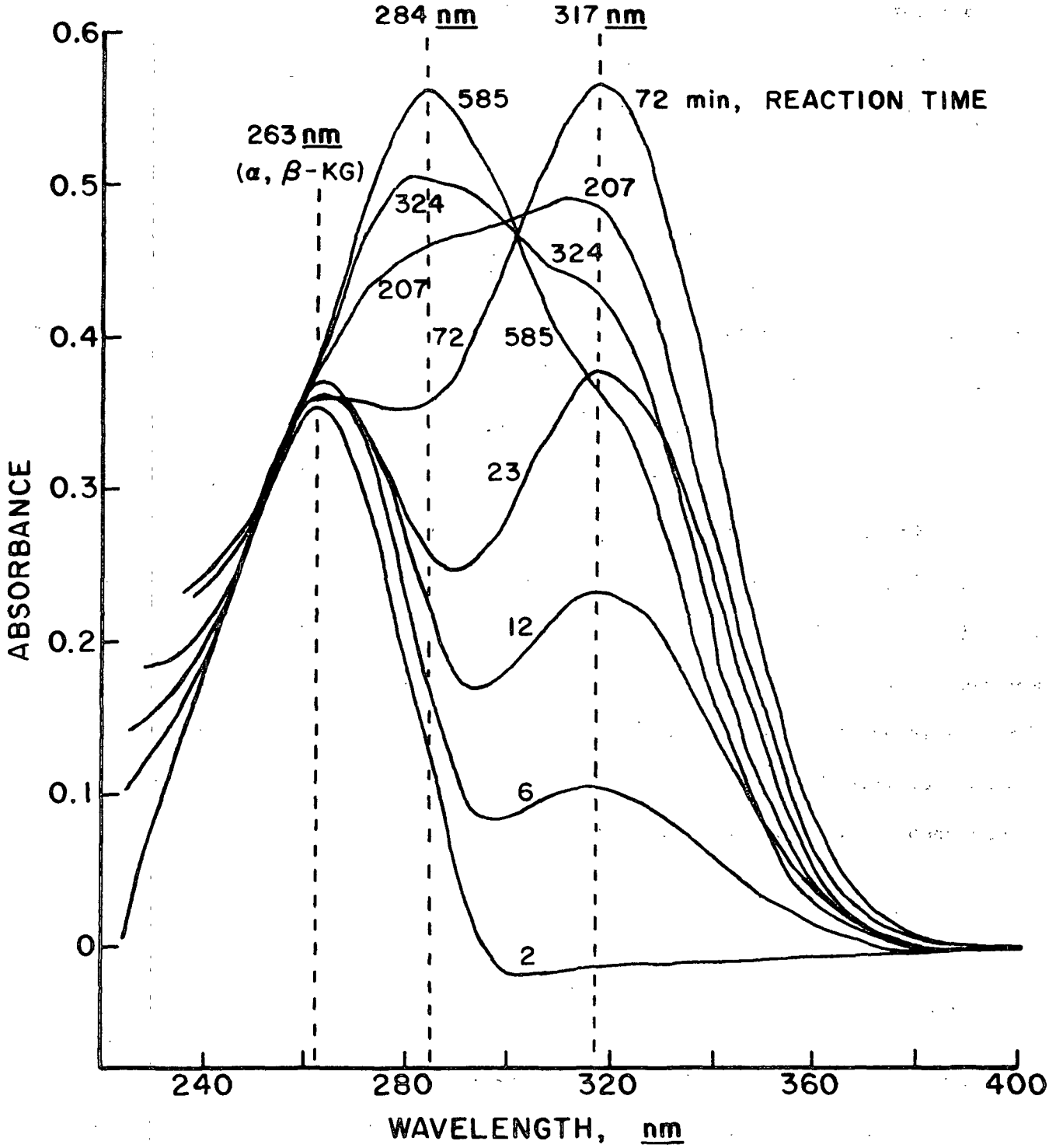


Figure 11. Change in Ultraviolet Absorption During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (ca. 8 x 10^{-5}M) in 0.05N NaOH

GLC Analysis

The products of the alkaline degradation of ET3M-KG and α,β-KG were analyzed by GLC as their per-O-trimethylsilyl (TMS) derivatives using ethyl 4,6-di-O-methyl-β-D-glycopyranoside as internal standard. The experimental conditions are described in the Experimental section, and the GLC conditions are outlined in Appendix I.

The nonvolatile products (products other than methanol and ethanol) in the alkaline degradation of ET3M-KG and α,β-KG were not very stable. α,β-KG which was the most stable of these products did not give a reproducible response even when it was analyzed on an all glass column.

Ethyl 3,4,6-tri-O-methyl-β-D-arabino-hexopyranosidulose reaction. The major initial nonvolatile product in this reaction was α,β-KG (Fig. 12). This compound was isolated by preparative GLC and analyzed by ^{1}H-NMR, ^{13}C-NMR, IR and UV spectroscopy. All of these spectra were identical with the corresponding spectra of known α,β-KG. The spectra are presented in Appendix V.

A very small amount of an unknown product (No. 1, Fig. 12) was isolated by preparative GLC, but its structure could not be determined. ^{1}H-NMR and IR spectra of unknown 1 are presented in Appendix V. The IR spectrum showed saturated C-H stretch and strong C-O-C bending vibrations but no carbonyl stretch nor unsaturated C-H stretch. The ^{1}H-NMR spectrum, which was poorly resolved, showed evidence for at least one TMS group and indicated that the ethyl aglycone was still intact. From this evidence, the product seemed to be an ethyl glycoside with at least one nonetherified hydroxyl. The origin of this type of product was indeterminate.

Ethyl 4-deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose reaction. The nonvolatile products from α,β-KG were so thermally unstable that they did

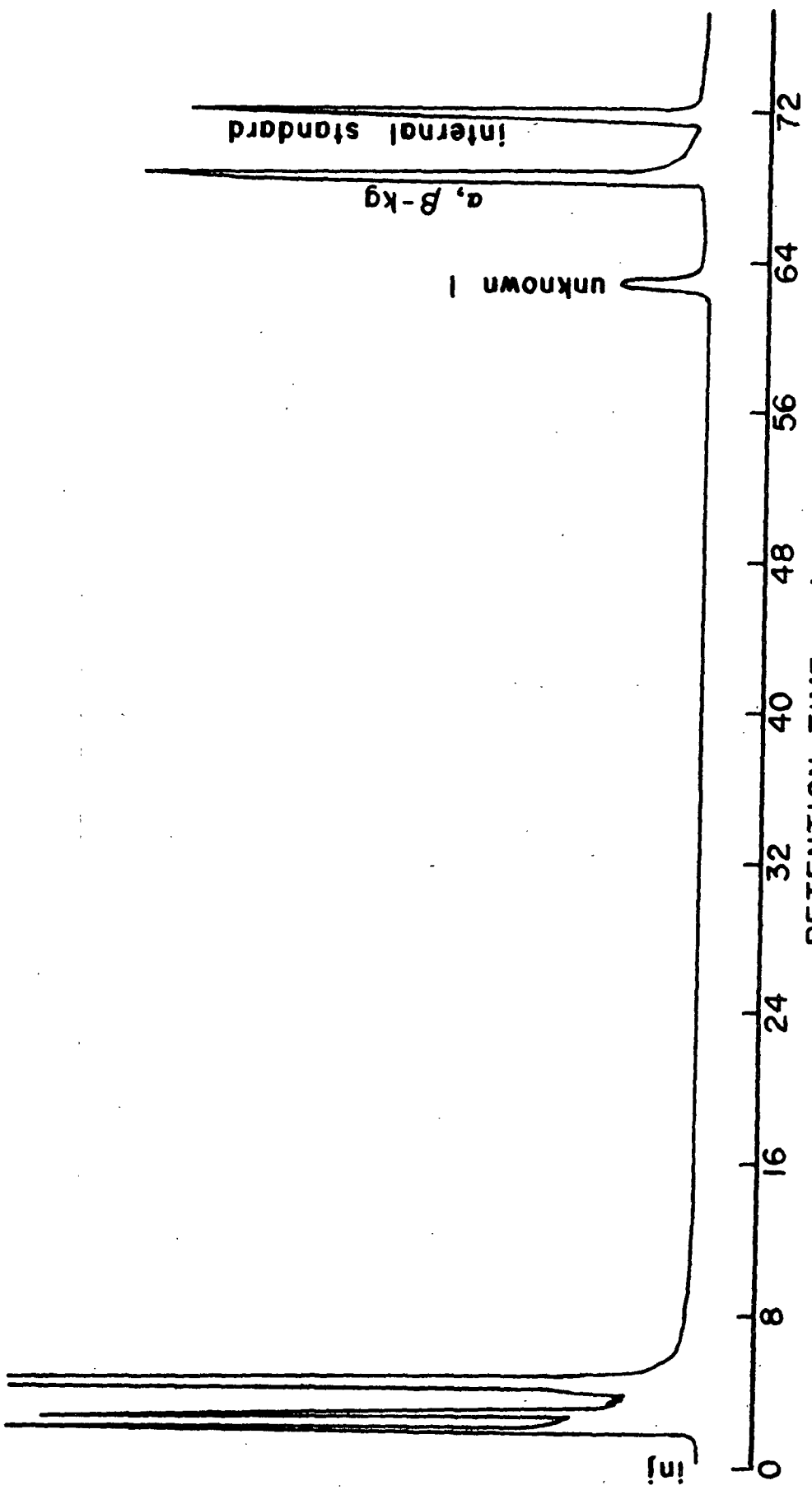
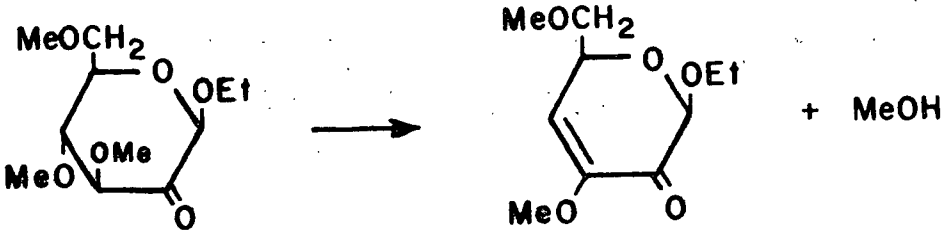


Figure 12. Sample Chromatogram of the Degradation Products of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH at 25°C (8 Minute Reaction Time). Chromatogram Obtained from Stainless Steel Column

not appear when the product mixture was analyzed on a stainless steel column. However, when an all glass column was used, six products were detected (Fig. 13). The only product that increased in concentration throughout the reaction was product "H" (Fig. 14). This product was isolated by preparative GLC. The structure of product "H" could not be determined. However, the infrared spectrum of "H", shown in Appendix V, had a strong broad O-H stretch (carboxyl hydroxyl group), strong C-H stretch, intermediate C=O stretch, strong COOH stretch, and strong C-O-C bend vibrations. This compound displayed a strong absorption maximum in the UV region at 284 nm (spectrum in Appendix V). ^{1}H-NMR spectra of product "H" were also taken but the spectra of two isolated samples were not reproducible. This evidence indicated that compound "H" was a keto-acid containing at least one ether group. The fact that "H" absorbed at 284 nm indicates that this probably was one of the terminal products in the alkaline reactions analyzed by UV spectroscopy.

PROPOSED ALKALINE DEGRADATION MECHANISM

It is generally accepted that keto-glycosides undergo rapid alkaline degradation by a beta-elimination mechanism (20,21,23,24,35). ET3M-KG initially degrades to α,β-KG, eliminating one mole of methanol. This initial step is supported by three independent pieces of evidence.



First, the unsaturated keto-glycoside (α,β-KG) was isolated and identified as the major nonvolatile product from the alkaline degradation of ET3M-KG. In

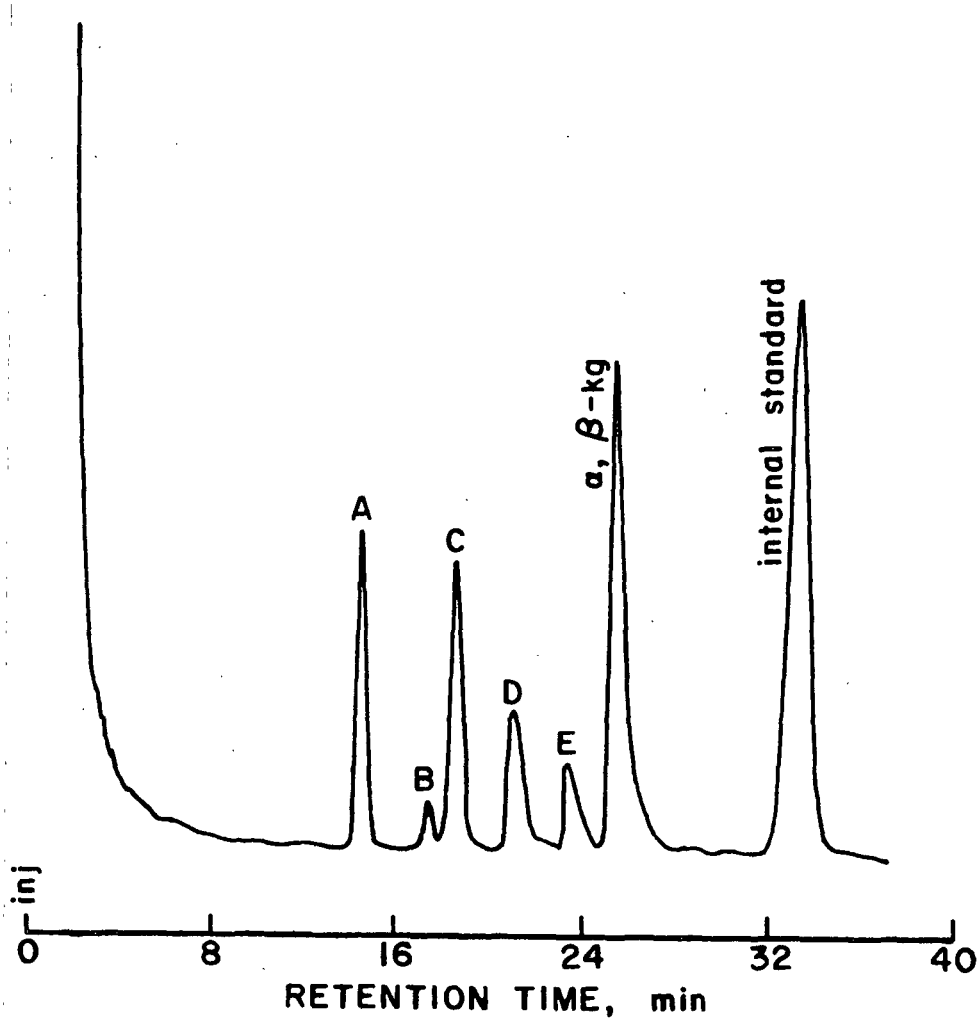


Figure 13. Sample Chromatogram of the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (0.10M) in 0.05N NaOH at 25°C (1.5 Minute Reaction Time). Chromatogram Obtained from a Glass Column. Products A Through E were Unidentified

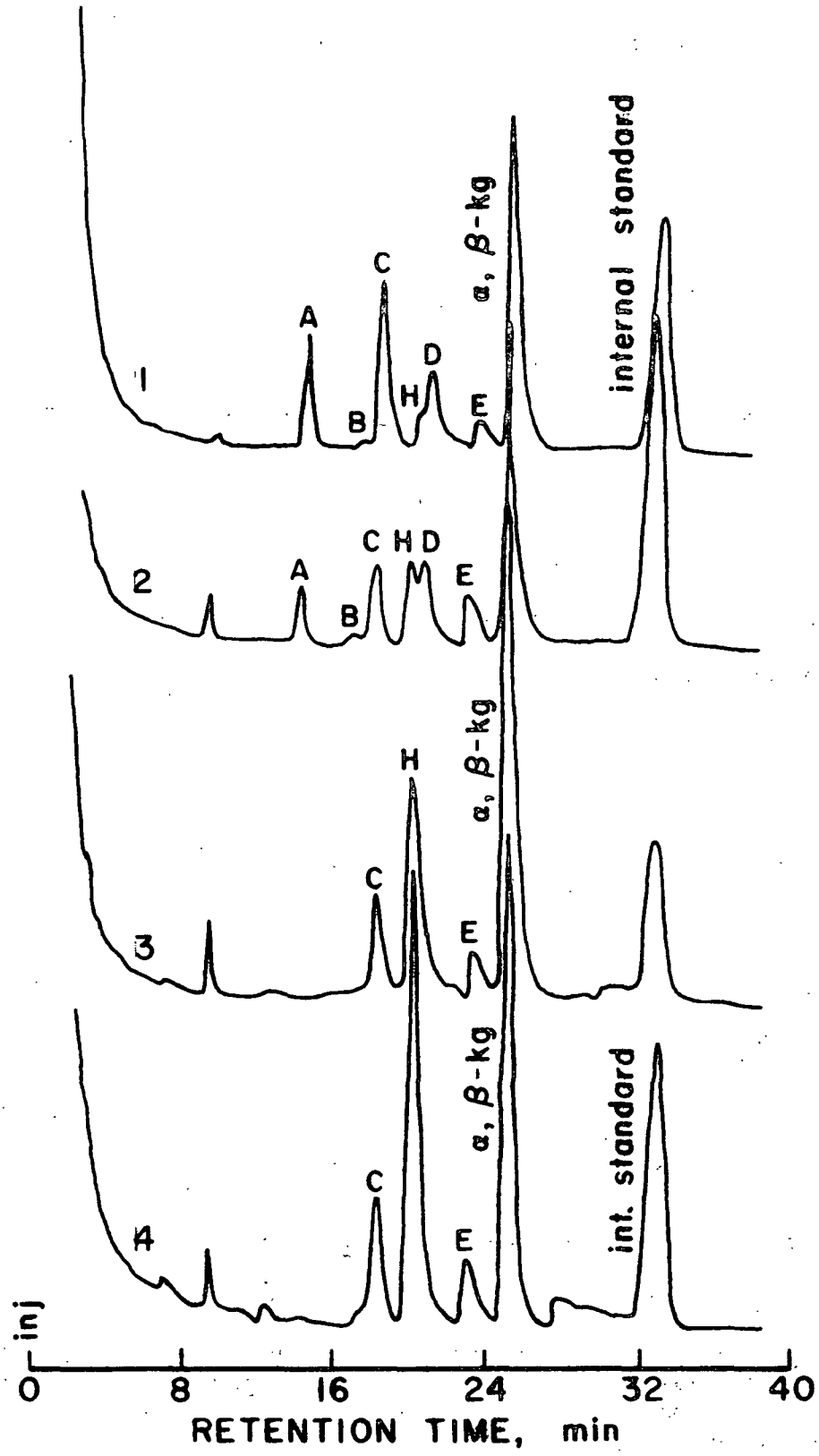


Figure 14. Sample Chromatograms of the Degradation Products of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (0.10M) in 0.05N NaOH at 25°C [(1) 4.0 Minutes, (2) 7.0 Minutes, (3) 18.0 Minutes, (4) 60.0 Minutes)]. Chromatograms Obtained from a Glass Column. Products A Through E were Unidentified. Concentrations were Measured Relative to the Internal Standard

addition, during the degradation the concentration of methanol liberated to the solution increased at a rate approximately equivalent to the rate of disappearance of ET3M-KG, implying that ET3M-KG degraded to α,β-KG by liberating 1 mole of methanol.

Second, ultraviolet analysis showed that when alkali was added to a dilute solution of ET3M-KG, an absorption maximum (263 nm) characteristic of α,β-KG was observed in the spectrum of the reaction solution within seconds (Fig. 11).

Finally, the observed optical rotation of a dilute solution of ET3M-KG ($[\alpha]_{546.1}$ -41.4°) initially decreased to approximately the rotation expected for a solution of the same concentration of α,β-KG ($[\alpha]_{546.1}$ -70.5°) before secondary reactions subsequently increased the observed rotation of the reaction solution (Fig. 15).

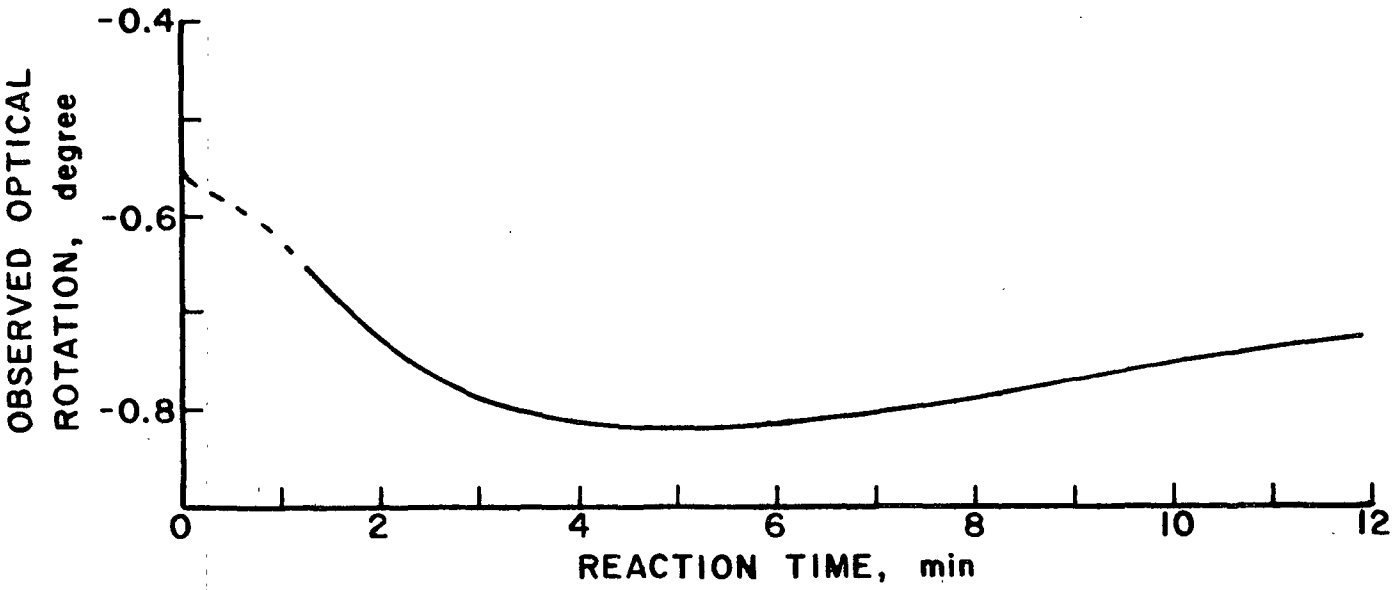


Figure 15. Change in Optical Rotation as a Function of Time During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.056M) in 0.05N NaOH at 25°C. Measurements were made using the 546.1 nm Line of the Mercury Lamp

The subsequent steps in this mechanism are more hypothetical since no other major products could be positively identified. As mentioned earlier, when ET3M-KG and α,β-KG were degraded in alkali, unidentified products that retained at least one of their methoxy substituents increased in concentration throughout the reaction. The mechanism proposed in Fig. 16 can be used to explain these observations.

In the proposed mechanism (Fig. 16) α,β-KG is degraded by an initial ring-opening beta-elimination reaction mechanism through a common carbanion to three α-keto esters, X, XI, and XII. α-Keto esters are known to be thermally unstable (36-38) yielding decarboxylation products when they are heated; and, therefore, this type of product may undergo surface catalyzed decomposition when product analyses were performed on a stainless steel GLC column. For this reason, α-keto esters, if they were present in the product mixture, would not be detected when a stainless steel GLC column was used for product analysis (refer to Fig. 13).

These keto esters could account for the absorption maximum at 317 nm observed in the UV spectrum of the alkaline ET3M-KG reaction solution. Going from α,β-KG to the α-keto ester X increases the conjugated enone system from 3 carbon atoms to 5. Such an increase in the conjugated system causes a bathochromic shift (shift in absorption to longer wavelength) from 263 nm to ca. 317 nm and a hyperchromic shift (intensity increases) (39,40) (refer to Fig. 11).

The optical rotation of esters X, XI, and XII should be zero, since they contain no asymmetric centers to rotate light. Therefore, as the α,β-KG slowly degrades in the alkaline solution, the optical rotation of the reaction solution would become more positive and would eventually reach zero if enough alkali were present to degrade all of the α,β-KG. This explains the behavior

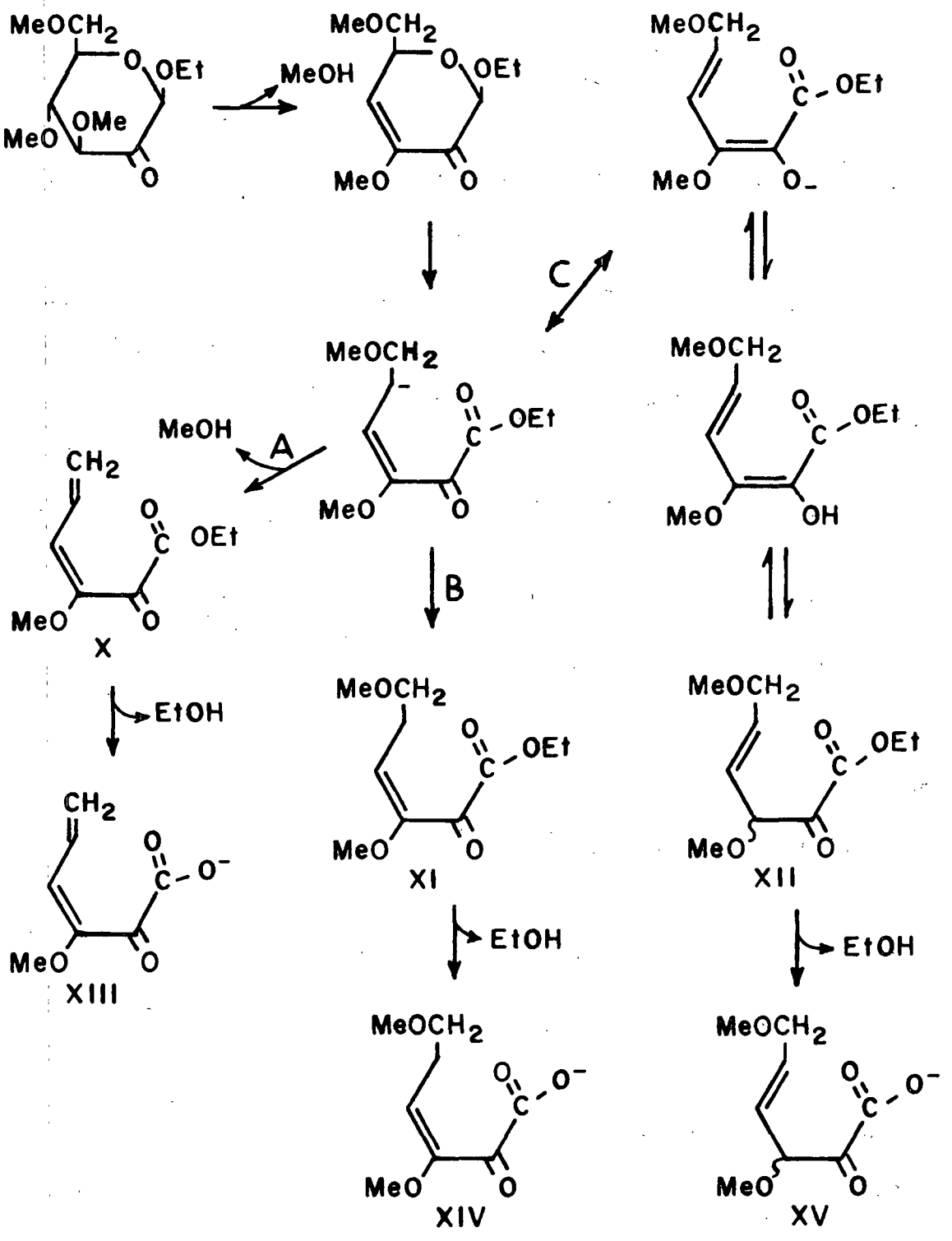


Figure 16. Proposed Mechanism for the Alkaline Degradation of ET3M-KG

observed during the alkaline degradation of ET3M-KG. In this reaction after an initial drop the optical rotation of the reaction solution slowly became more positive (Fig. 15).

In alkaline solutions, ethyl esters would be saponified to carboxylic acid salts and ethanol. Therefore, the alkali consumed in these reactions is probably due to the saponification of ethyl esters such as X, XI, and XII.

The shift in absorption maxima on going from α-keto esters such as X, XI, and XII to α-keto carboxylic acid salts like XIII, XIV, and XV is not usually more than 2 nm (39). If the absorption maximum at 317 nm was due to the α-keto ester X and the α-keto acid XIII, then the absorption maximum at 284 nm could result from the formation of a product containing one less double bond in its conjugated system. This could be accomplished by the reaction sequence presented in Fig. 17. The hydroxide ion, functioning as a nucleophile, could add to the conjugated system of XIII to form XVI (Fig. 17), which could undergo a reverse aldo condensation to form the α-keto acid, XVII. The α-keto acid (XVII) should display a hypsochromic shift (shift to lower wavelength) of 30 nm from XIII (39). It should also absorb at a higher wavelength than α,β-KG, since the carboxylate anion would contribute slightly to the conjugated system.

In the alkaline degradation of α,β-KG, more ethanol was liberated than methanol (Fig. 8). α,β-KG contains one mole of ethanol and 2 moles of methanol. It is evident from Table II and Fig. 10 that every equivalent of α,β-KG that degraded in the alkaline solution released one equivalent of ethanol. After 60 minutes of reaction the methanol that was liberated during this reaction amounted to only 70% of the ethanol that was liberated (refer to Fig. 8). This indicates that 70% of the degradation proceeded *via* a pathway such as A (Fig. 16) in which a product that retained only one of its methoxyl groups (XVII) is

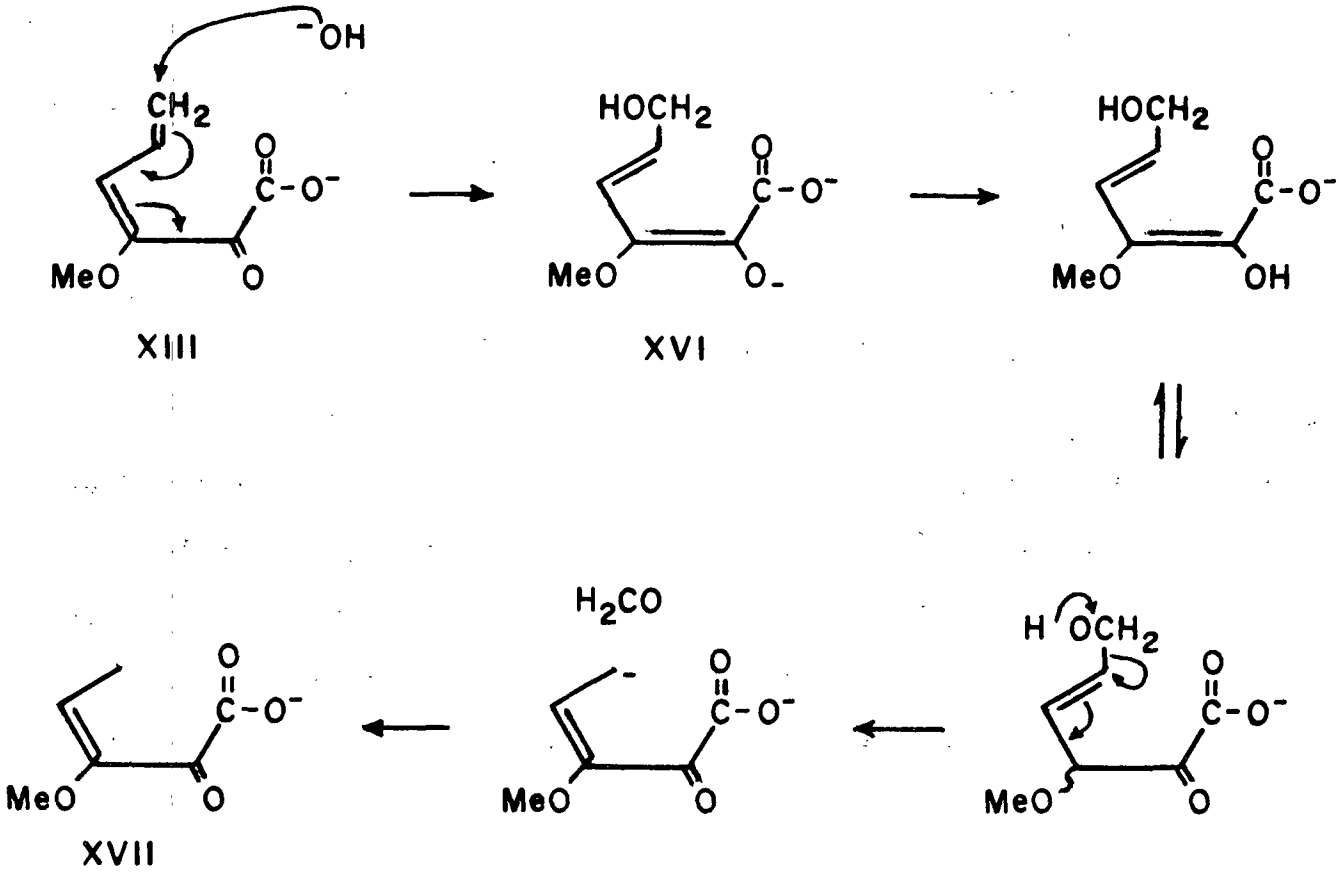


Figure 17. Degradation of Compound XIII via Reverse Aldo-Condensation to Form XVII

formed. The remaining 30% of the degradation would then proceed through pathways such as B and C (Fig. 16), which would yield products that retained both of their methoxy substituents.

As mentioned earlier, the IR spectrum of product "H" indicated that it was a keto acid containing at least one alkyl ether group (Fig. 43, Appendix V). This product increased in concentration throughout the reaction and was by far the major degradation product (Fig. 14). Since it would be a terminal product of pathway A, XVII is a possible structure for product "H", which by virtue of the alkoxy substituent balances, could account for 70% of the product mixture. However, although structure XVII is consistent with the IR and UV spectra of "H" (Fig. 43 and 44, Appendix V), other structures can also be presented that would be consistent with these spectra.

ALKALINE PEROXIDE REACTIONS

INTRODUCTION

ET3M-KG (0.1M) was degraded at 25°C in 0.05N sodium hydroxide and 0.1M hydrogen peroxide in passivated glass reaction flasks. No attempt was made to ensure that the reactions were run under an inert atmosphere. Reaction samples were quenched with sodium borohydride which reduced unreacted keto-glycoside and residual hydrogen peroxide. The epimeric glycosides resulting from the reduction of ET3M-KG were analyzed by GLC in the same manner used in the alkaline reactions.

The concentration of hydrogen peroxide was measured by a titanium sulfate colorimetric method (1,2,6,7,22,41-43). Titanium(IV) forms a stable, colored complex with hydrogen peroxide in strongly acidic solution. In addition, the strongly acidic solutions can hydrolyze most organic peroxides, at varying

rates, to form hydrogen peroxide, which would be available to complex with the titanium(IV) cation.

Hydrogen peroxide complexes immediately with the titanium(IV) cation; therefore, the initial absorbance of the yellow complex could be related to the hydrogen peroxide concentration through a calibration curve. Organic peroxide concentrations could be determined from increases in this absorption with time. Procedures used for these measurements are outlined in the Experimental section.

ET3M-KG underwent a rapid initial degradation in alkaline peroxide solution; during this time the concentration of α,β-KG built up rapidly (Fig. 18). In contrast to the alkaline degradation reactions, this reaction solution did not become yellow when the alkaline peroxide solution was added to the ET3M-KG, indicating that conjugated chromophores were not formed in this reaction. The rapid initial degradation was followed by a regime in which little degradation of ET3M-KG occurred. In this regime the concentration of ET3M-KG did not change for up to 7 days, indicating that the reaction had come to a complete halt after the first 15 minutes.

Since α,β-KG was the major product of the reaction, it was desirable to study its behavior in alkaline peroxide solution. Therefore, α,β-KG was degraded at 25°C in a solution of 0.05N sodium hydroxide and 0.1M hydrogen peroxide. The reaction was performed under conditions identical to the alkaline peroxide degradation of ET3M-KG. Reaction samples were treated identically to the way they were treated in the alkaline degradation of α,β-KG.

α,β-KG initially underwent a relatively rapid degradation followed by a period of much slower degradation (Fig. 19). Like the alkaline peroxide degradation of ET3M-KG, the reaction solution did not become yellow when the alkaline peroxide solution was added to the α,β-KG.

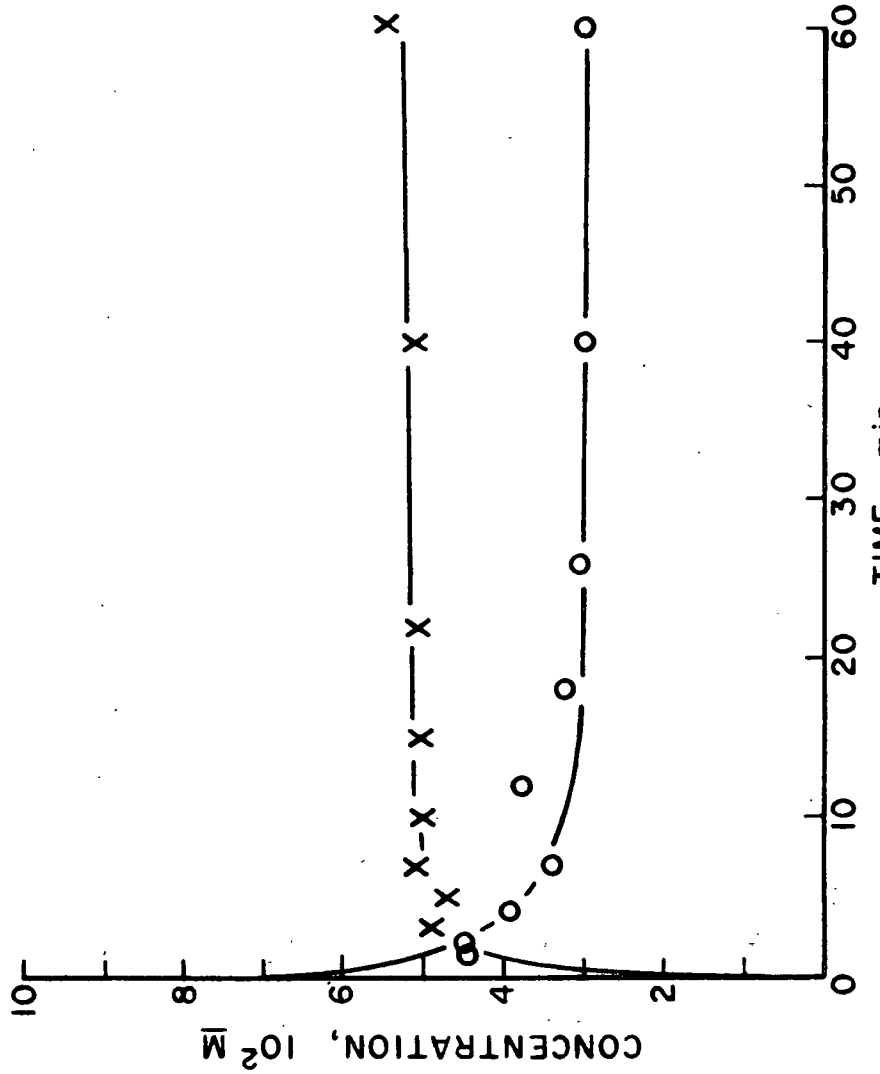


Figure 18. Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (O) and Formation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (x) in 0.05N NaOH and 0.10M H_2O_2 at 25°C

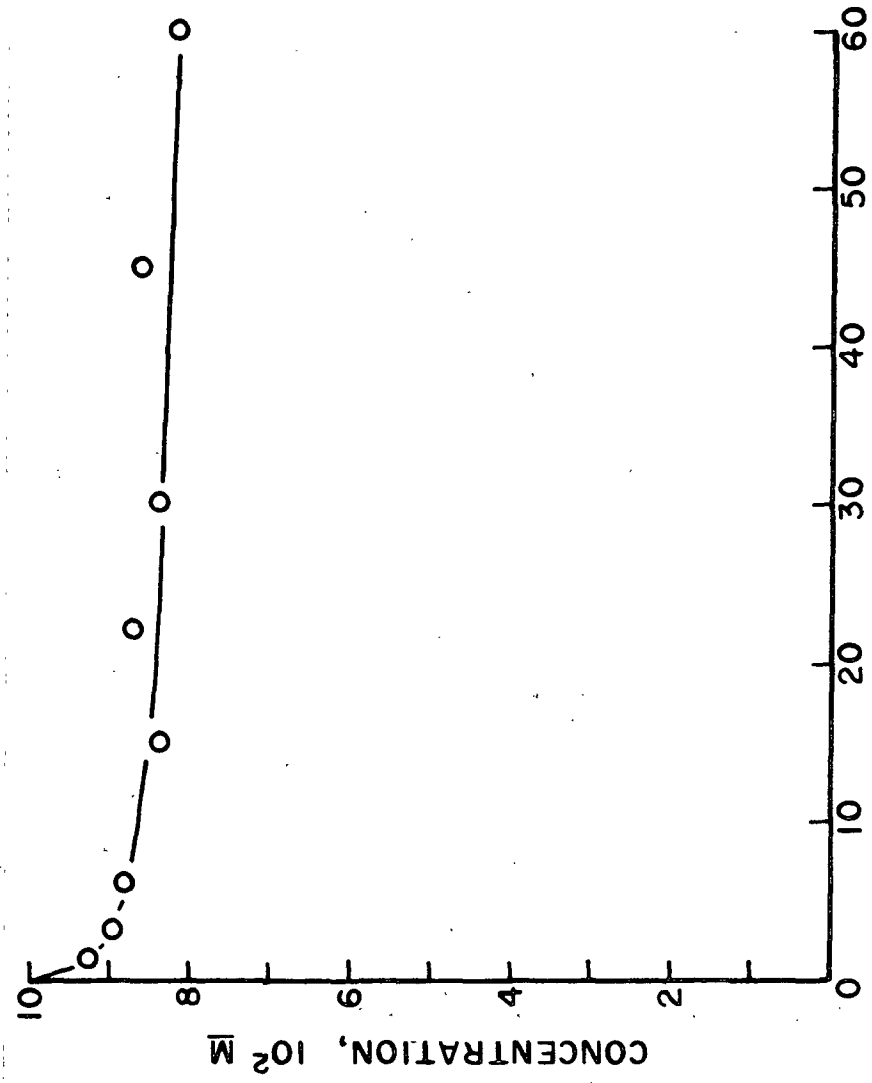


Figure 19. Degradation of Ethyl 4-Deoxy 3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose in 0.05N NaOH and 0.10M H_2O_2 at 25°C

ALKALI CONSUMPTION

Although the alkali charge in the alkaline peroxide reactions was the same as in the alkaline reactions, the initial available hydroxide ion concentration, as determined by pH measurement, was ten times lower. This was because hydrogen peroxide, acting as a weak acid, removed 90% of the hydroxide ion from solution by its ionization to the hydroperoxide anion. (See Appendix IV for available hydroxide concentration calculations.)

$$HOOH + OH^- \rightleftharpoons HOO^- + H_2O$$

During the alkaline peroxide degradation of ET3M-KG (Fig. 20) and α,β-KG (Fig. 21), the alkali was rapidly consumed by acidic degradation products that were generated during the reaction. Figures 20 and 21 show that after 10 minutes of reaction, essentially all of the available hydroxide ion had been consumed. At the same time in the reaction, the rate of ET3M-KG degradation slowed considerably (Fig. 18), suggesting that the reaction slowed and eventually stopped because the reaction solution was depleted of alkali.

To investigate this hypothesis, two reactions of ET3M-KG were performed at constant hydroxide ion concentration. The first reaction was performed in a solution buffered to pH 8.0, which was the pH of the unbuffered alkaline peroxide reaction solution after 1 hour of reaction. The second reaction was carried out in a solution buffered to pH 9.5, the pH of the unbuffered reaction solution when ET3M-KG degradation started to slow down. There was no degradation of ET3M-KG in either of these reactions 1 hour after the reagents were mixed.

The lack of reaction in these buffered solutions strongly indicated that the decrease in reactivity of ET3M-KG observed during the alkaline peroxide

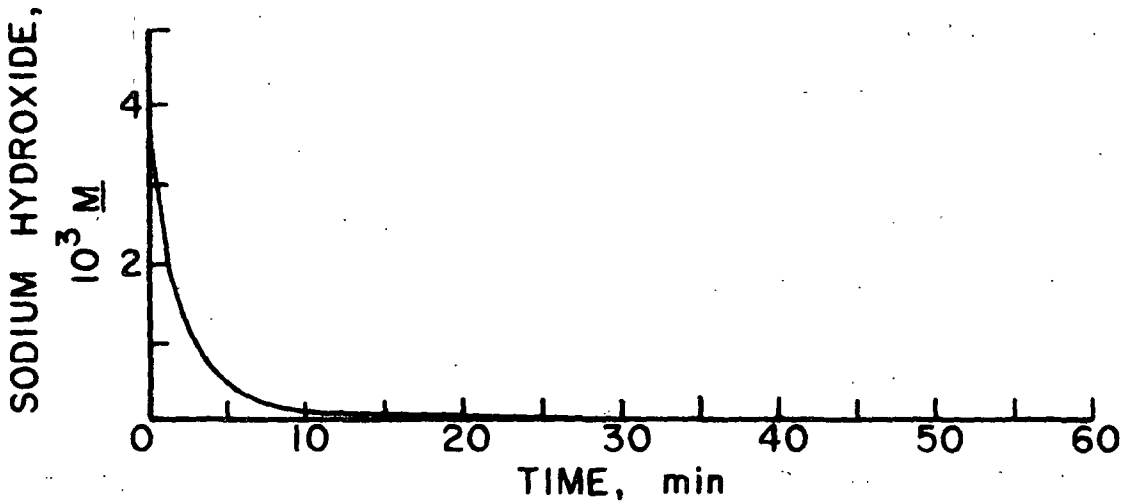


Figure 20. Change in Alkali Concentration During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH and 0.10M H_2O_2 at 25°C

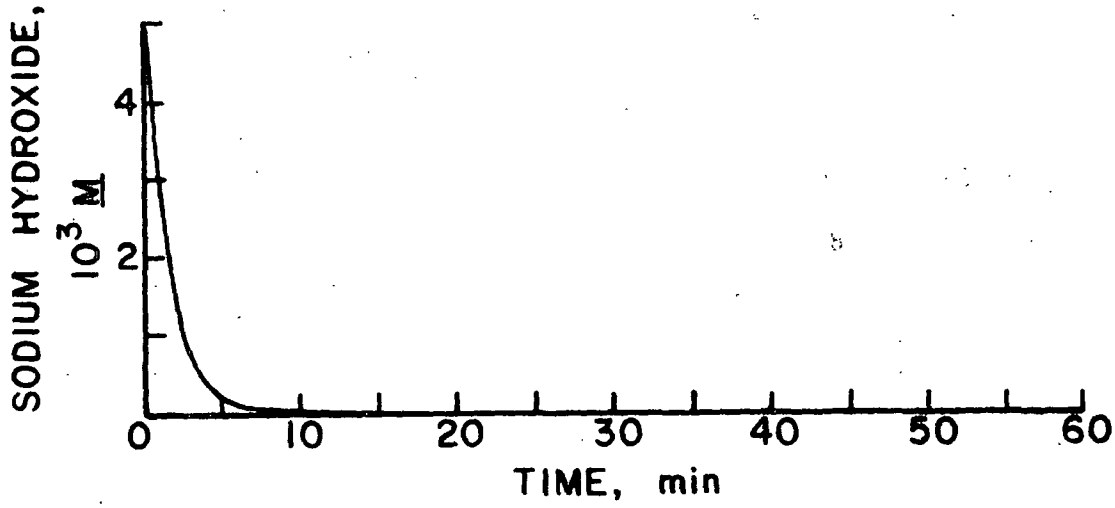


Figure 21. Change in Alkali Concentration During the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (0.10M) in 0.05N NaOH and 0.10M H_2O_2 at 25°C

reaction was indeed due to depletion of alkali caused by acidic products generated during the reaction.

To further test this hypothesis, a reaction of ET3M-KG in a solution of 0.25N sodium hydroxide and 0.1M hydrogen peroxide at 25°C was performed. This reaction was identical to the previously reported alkaline peroxide degradation reaction of ET3M-KG in other respects, except that the alcohol concentrations were not followed with time. The α,β-KG formation was obtained by GLC analysis of the reduced species by assuming a detector response factor of one.

In this reaction, ET3M-KG degraded rapidly and completely (Fig. 22). On addition of the alkaline peroxide solution to the ET3M-KG, the reaction solution immediately became yellow much like the reaction solutions of the alkaline degradations. The "concentration" of α,β-KG increased to approximately a 25% yield*, then slowly degraded until it had all disappeared after 60 minutes of reaction.

The hydrogen peroxide was rapidly and completely consumed in the reaction (Fig. 22). However, when a reaction solution was prepared with the same concentration of hydrogen peroxide and sodium hydroxide but with no organic substrate, the hydrogen peroxide was stable for at least 3 hours, implying that the hydrogen peroxide in the alkaline peroxide degradation reaction of ET3M-KG was used to oxidize the products of degradation. Only minor amounts of organic peroxides were formed in this reaction.

*This yield was calculated using a response factor for α,β-KG of 1.

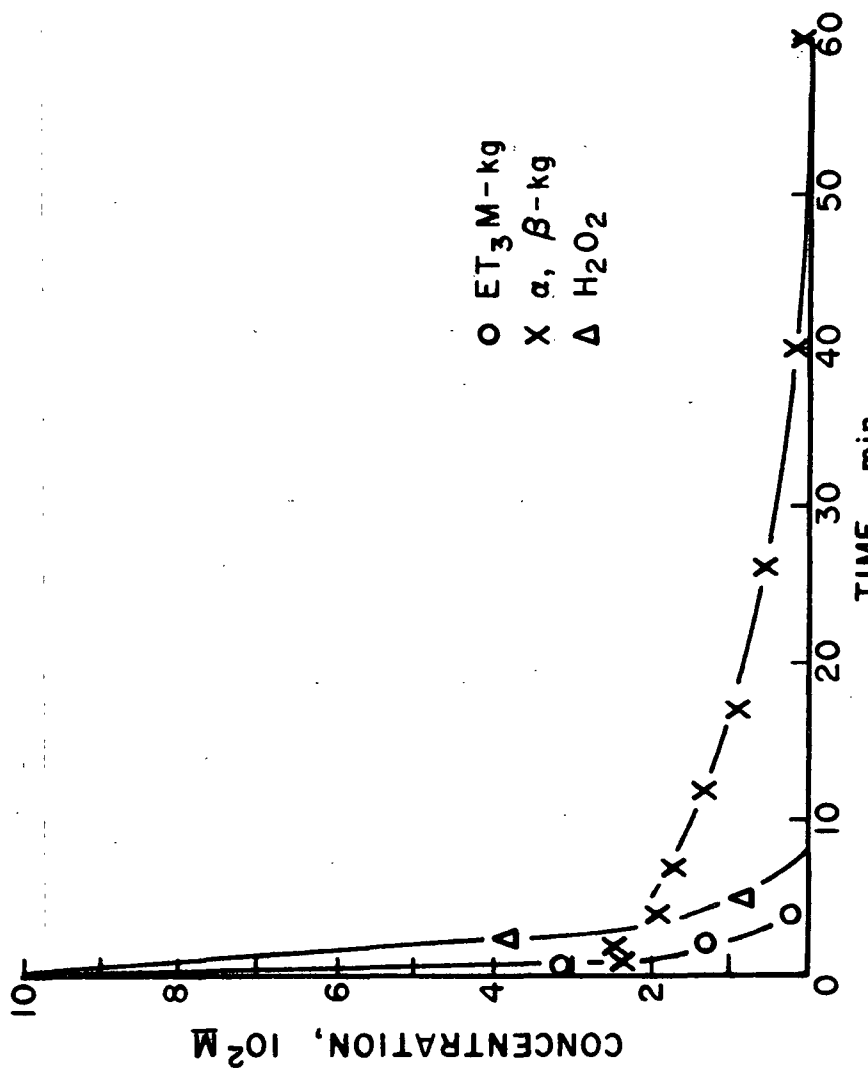


Figure 22. Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose and Formation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose in 0.25N NaOH and 0.10M H_2O_2 at 25°C

PEROXIDES

Hydrogen Peroxide

During the initial phase of the ET3M-KG alkaline peroxide reaction, hydrogen peroxide was rapidly consumed (Fig. 23). After the ET3M-KG degradation leveled off, the hydrogen peroxide consumption slowed down.

During the initial phase of α,β-KG degradation, hydrogen peroxide was consumed much more rapidly than α,β-KG (Fig. 24). In this reaction the hydrogen peroxide concentration leveled off at approximately the same value that it did in the alkaline peroxide degradation of ET3M-KG, at which time approximately 3 moles of hydrogen peroxide were consumed for each mole of α,β-KG.

Organic Peroxides

During the alkaline peroxide reactions, minor amounts of organic peroxides which appeared to reach a maximum concentration at early reaction times were formed (Fig. 22 and 23). The concentration of organic peroxides was barely significant when measured over a relatively large concentration of residual hydrogen peroxide.

Organic peroxides, reportedly α-hydroxyhydroperoxides, are readily formed when common ketones, such as cyclohexanone and acetone, are placed in aqueous solutions of hydrogen peroxide (3-5). Therefore, ET3M-KG and α,β-KG were dissolved in aqueous solutions of 0.1M hydrogen peroxide at 25°C. The reaction was performed in passivated reaction glassware at a keto-glycoside concentration of 0.1M. The pH of the reaction solution was ca. 6. In both cases the concentration of hydrogen peroxide remained unchanged over 1 hour, and no significant amounts of organic peroxides were formed (Appendix III). This indicated that in the case of ET3M-KG and α,β-KG, the equilibrium between the ketone and the α-hydroxyhydroperoxide is far toward the ketone.

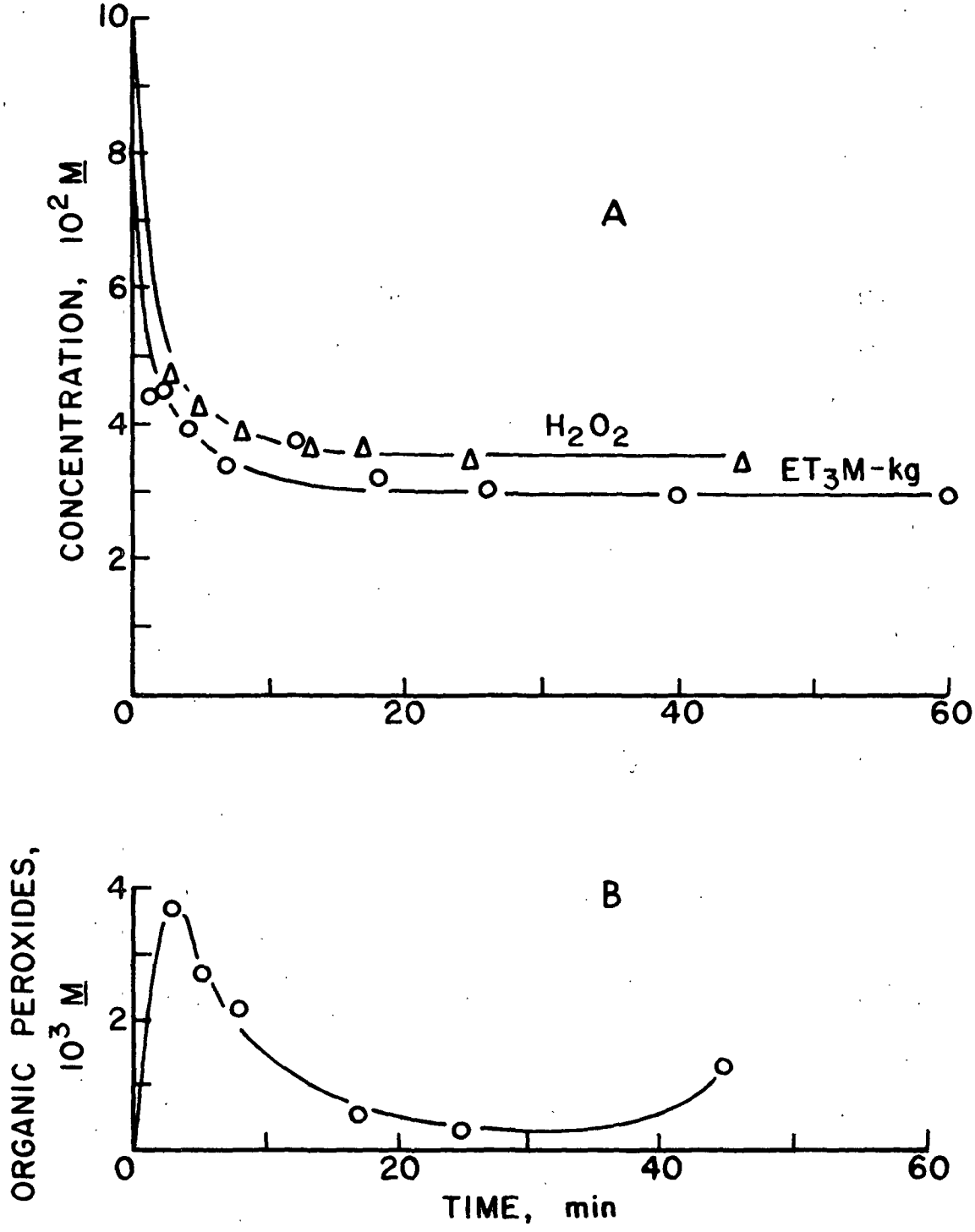


Figure 23. Consumption of Hydrogen Peroxide (A) and Formation of Organic Peroxides (B) During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose in 0.05N NaOH and 0.10M H_2O_2 at 25°C

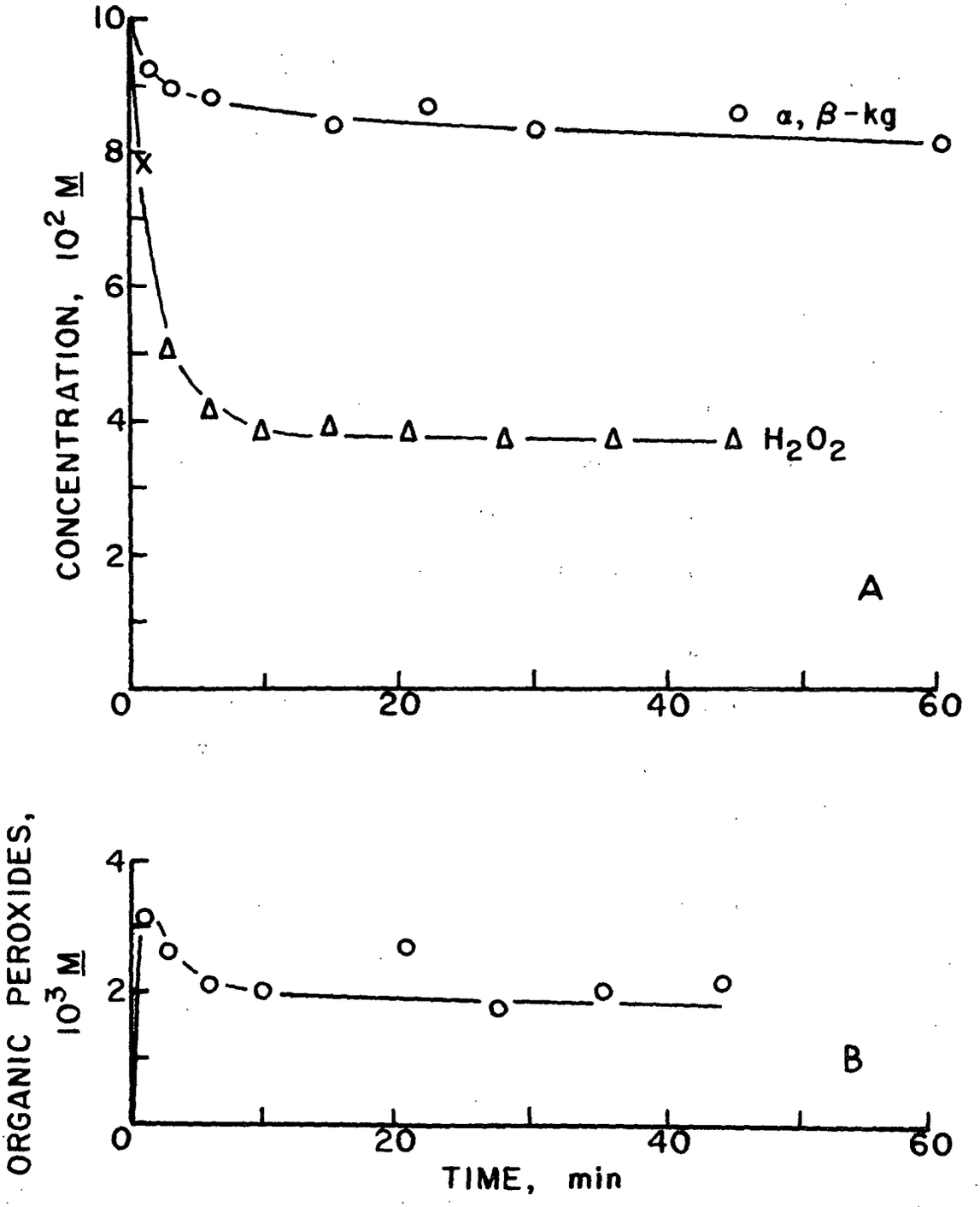
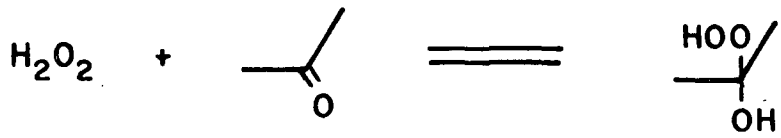


Figure 24. Consumption of Hydrogen Peroxide (A) and Formation of Organic Peroxides (B) During the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose in 0.05N NaOH and 0.10M H_2O_2 at 25°C



PRODUCTS

Alcohols

The amount of methanol and ethanol formed in the alkaline peroxide reactions was much less than that formed in the corresponding alkaline degradation reaction. Obviously this was due in part to the lower available alkali concentration at which the alkaline peroxide reactions were run.

In the alkaline peroxide reaction of ET3M-KG, the formation of methanol was rapid during the initial portion of the reaction (Fig. 25). Ethanol was formed at a much slower rate and both alcohols leveled off after the ET3M-KG had stopped reacting.

In the alkaline peroxide degradation of α,β-KG, again methanol was formed very rapidly during the initial phase of the reaction (Fig. 26). Ethanol was formed at a slower rate and leveled off at about half of the concentration of methanol.

Molar balances on the alkoxy groups of the reacting keto-glycosides (Tables III and IV) were constructed in the same manner as outlined under the section on alkaline reactions, and are graphically displayed in Fig. 27 and 28.

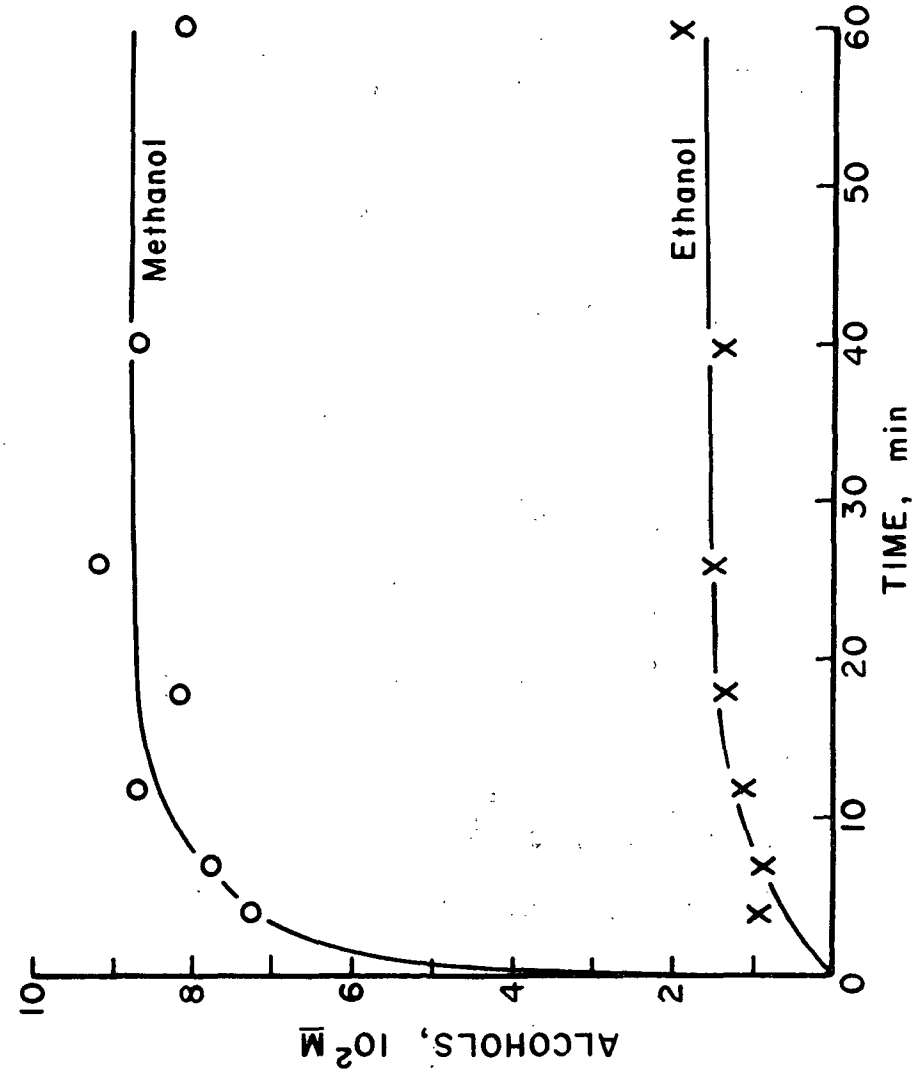


Figure 25. Formation of Methanol (○) and Ethanol (X) During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH and 0.10M H_2O_2 at 25°C

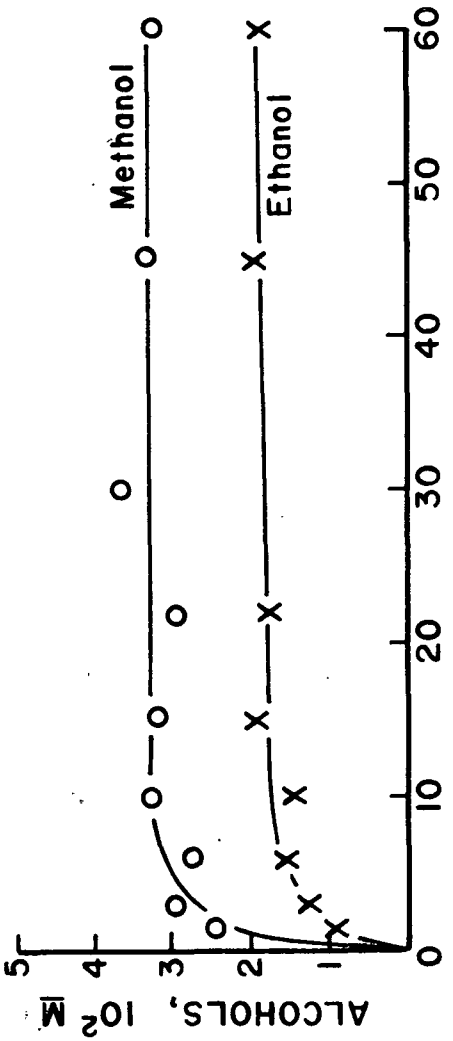


Figure 26. Formation of Methanol (○) and Ethanol (X) During the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (0.10M) in 0.05N NaOH and 0.10M H_2O_2 at 25°C

TABLE III

MOLAR BALANCE ON ALKOXY SUBSTITUENTS FOR THE ET3M-KG ALKALINE PEROXIDE DEGRADATION

Reaction Time, min	ET3M-KG x 10^2M	α,β-KG x 10^2M	MeOH x 10^2M	EtOH x 10^2M	Total $\overline{MeO}$ x 10^2M	Total $\overline{EtO}$ x 10^2M	Molar $\overline{MeO}$, %	Molar $\overline{EtO}$, %
0.0	0.100	--	--	--	0.300	0.100	100.0	100.0
1.0	0.058	0.042	0.026	0.003	0.283	0.102	94.3	102.0
2.0	0.044	0.046	0.058	0.005	0.282	0.095	94.0	95.0
4.0	0.040	0.048	0.070	0.008	0.285	0.095	95.0	95.0
8.0	0.035	0.050	0.078	0.010	0.284	0.096	94.7	96.0
12.0	0.033	0.051	0.082	0.013	0.281	0.096	93.7	96.0
15.0	0.032	0.051	0.083	0.013	0.281	0.096	93.7	96.0
20.0	0.031	0.052	0.085	0.015	0.281	0.097	93.7	97.0
40.0	0.030	0.053	0.087	0.016	0.282	0.098	94.0	98.0
60.0	0.030	0.053	0.088	0.017	0.283	0.100	94.3	100.0

TABLE IV

MOLAR BALANCE ON ALKOXY SUBSTITUENTS FOR THE α,β-KG ALKALINE PEROXIDE DEGRADATION

Reaction Time, min	α,β-KG x 10^2M	MeOH x 10^2M	EtOH x 10^2M	Total $\overline{MeO}$ x 10^2M	Total $\overline{EtO}$ x 10^2M	Molar $\overline{MeO}$, %	Molar $\overline{EtO}$, %
0.0	0.100	--	--	0.200	0.100	100.0	100.0
1.0	0.093	0.018	0.007	0.205	0.101	102.5	101.0
2.0	0.091	0.025	0.010	0.208	0.101	104.0	101.0
4.0	0.088	0.028	0.013	0.205	0.101	102.5	101.0
8.0	0.088	0.030	0.015	0.205	0.103	102.5	102.0
12.0	0.087	0.031	0.016	0.205	0.103	102.5	103.0
16.0	0.086	0.032	0.017	0.204	0.103	102.0	103.0
20.0	0.085	0.032	0.018	0.203	0.103	101.5	103.0
25.0	0.085	0.032	0.019	0.202	0.103	101.0	103.0
35.0	0.084	0.033	0.019	0.200	0.103	100.0	103.0
45.0	0.083	0.033	0.020	0.198	0.103	99.0	103.0
60.0	0.082	0.034	0.020	0.198	0.102	99.0	102.0

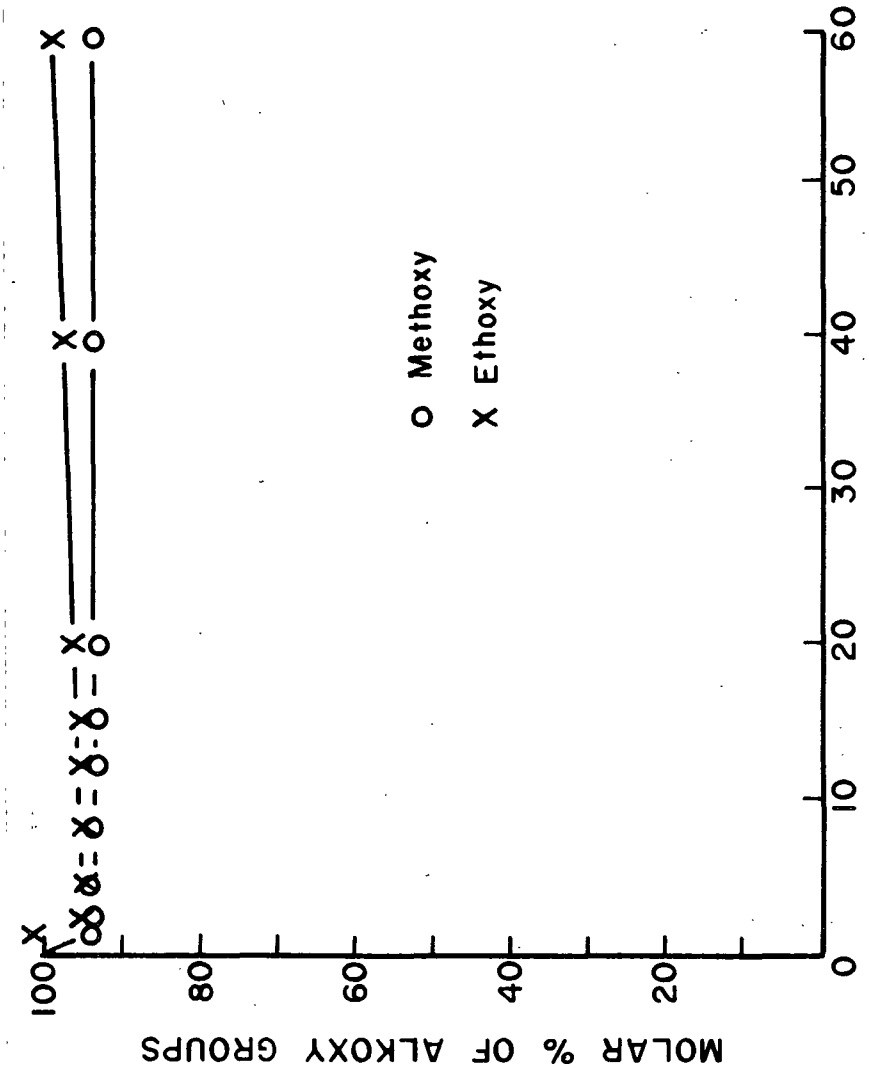


Figure 27. Percentage of Alkoxy Groups Accounted for During the Degradation of Ethyl 3,4,6-Tri-O-methyl-3-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH and 0.10M H_2O_2 at 25°C

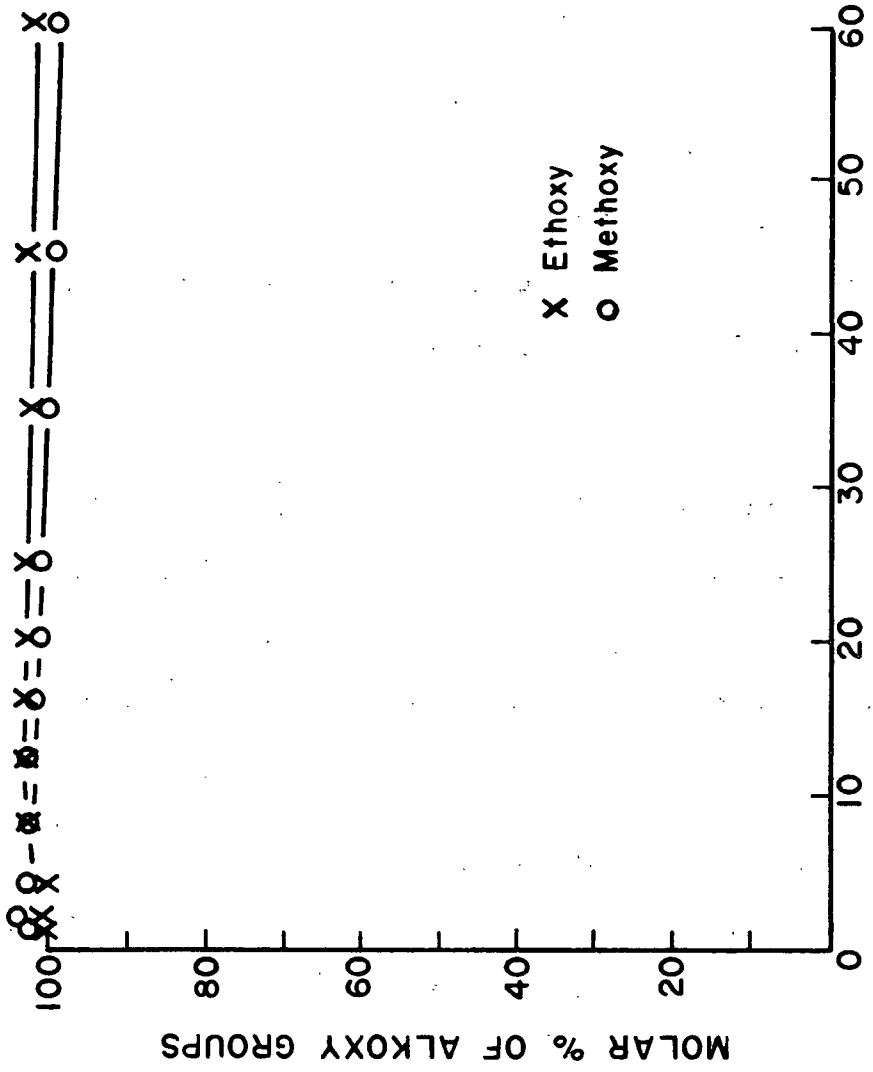


Figure 28. Percentage of Alkoxy Substituents Accounted for During the Degradation of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (0.10M) in 0.05N NaOH and 0.10M H_2O_2 at 25°C

In the alkaline peroxide degradation of ET3M-KG, the molar percentages of methoxy groups and ethoxy groups remained constant throughout the entire reaction (Table III and Fig. 27); this indicates that no unanalyzed reaction products, which retained any of their alkoxy substituents, were stable to the reaction conditions. The data in Table III were compiled from several reactions. Although it was determined that these reactions displayed excellent reproducibility, the deviation of the data from 100% could be attributed to experimental error.

Table IV and Fig. 28 show that in the alkaline peroxide degradation of α,β-KG, all of the methoxy and ethoxy substituents could be accounted for throughout the entire reaction. Therefore, there were no stable unanalyzed methoxy- or ethoxy-containing products in this reaction either.

Other Products

α,β-KG was identified as the major nonvolatile product in the alkaline peroxide degradation of ET3M-KG. The concentration of α,β-KG throughout the reaction was obtained by a colorimetric UV analysis, since analysis by GLC led to an erratic concentration profile. Analytical details are given in the Experimental section.

The concentration of α,β-KG increased rapidly during the initial phase of ET3M-KG degradation (Fig. 18), and leveled off when ET3M-KG degradation slowed down. Presumably, unlike during the alkaline degradation of ET3M-KG, the pH in this reaction was not high enough to cause appreciable alkaline degradation of α,β-KG once it was formed.

No other nonvolatile products generated during the alkaline peroxide degradation of α,β-KG could be detected by GLC analysis of product samples even when the analysis was performed on an all glass column. A mixture of highly

polar, nonvolatile products was obtained from the baseline of the thin-layer chromatoplates used for kinetic analysis of α,β-KG during this reaction. The mixture had the following composite IR spectrum: a strong broad -OH stretching absorption between 3700 and 2200 cm^{-1}, a series of -C-H stretching maxima between 2950 and 2850 cm^{-1}, an intermediate carbonyl maximum at 1730 cm^{-1} and strong -COO stretching at 1660 cm^{-1} and 1580 cm^{-1}; its UV spectrum showed no absorption above 230 nm. This evidence implied that these polar compounds were carboxylic acids containing no conjugated chromophores.

PROPOSED ALKALINE PEROXIDE DEGRADATION MECHANISM

The initial step in the alkaline degradation of ET3M-KG was not affected by the addition of hydrogen peroxide. α,β-KG was isolated and characterized as the first formed product in the alkaline peroxide degradation of ET3M-KG. The formation of methanol paralleled the rapid formation of α,β-KG.

The role that hydrogen peroxide played in the degradation mechanism began to unravel when α,β-KG was degraded under conditions that were identical to the alkaline peroxide degradation of ET3M-KG. In this reaction, three equivalents of hydrogen peroxide were consumed for each equivalent of α,β-KG that reacted. Hydrogen peroxide was consumed rapidly in both the ET3M-KG and α,β-KG alkaline peroxide reactions and the amount of hydrogen peroxide consumed in these two reactions was approximately equal.

Analysis of the ET3M-KG reaction solution by UV spectroscopy afforded another insight into the effect of hydrogen peroxide on the alkaline degradation mechanism. The only chromophore that was apparent throughout the reaction was the one characteristic of α,β-KG. The highly conjugated products proposed in the alkaline degradation mechanism, which exhibited strong UV absorption maxima, were not formed in the alkaline peroxide degradation of ET3M-KG.

The molar balance of alkoxy substituents (Tables III and IV) showed that in these reactions, all of the methoxy and ethoxy substituents initially present in the reacting keto-glycoside could be accounted for throughout the entire reactions. This indicated that as the keto-glycoside degraded no products retaining methoxy or ethoxy substituents were formed.

The mechanism proposed for alkaline peroxide degradation of ET3M-KG in Fig. 29 is consistent with these observations. As mentioned previously, the first step in this mechanism is the same as in the alkaline degradation reaction. The subsequent steps involve a Michael addition of the nucleophilic hydroperoxide anion to the conjugated double bond, resulting in the β-hydroperoxy ketone (XVIII). The hydroperoxy ketone (XVIII) can undergo a base-catalyzed epoxidation reaction (31-33) to form an α,β-epoxy ketone (XIX), which can subsequently undergo a base-catalyzed, ring opening elimination reaction to yield an α-keto ester (XX). This ester (XX) will liberate ethanol by saponification to the carboxylic acid salt, XXI. The hydroperoxide anion, acting as the strongest nucleophile in the system, can open the epoxide ring to form an alkali-labile hemiketal (XXII), which would rapidly lose methanol to form an α-diketone, XXIII. A third mole of hydrogen peroxide adds to the α-diketone and eventually leads to carbon-carbon bond cleavage that results in XXIV and the oxalate anion.

Although alternative mechanisms can be proposed, the reactions presented in Fig. 29 best satisfy the experimental results obtained in this work.

The one experimental finding that is not consistent with the proposed mechanism is the low level of organic peroxides that was measured in these reactions. If the α-hydroperoxy acid (XXIV) is a stable product, then it

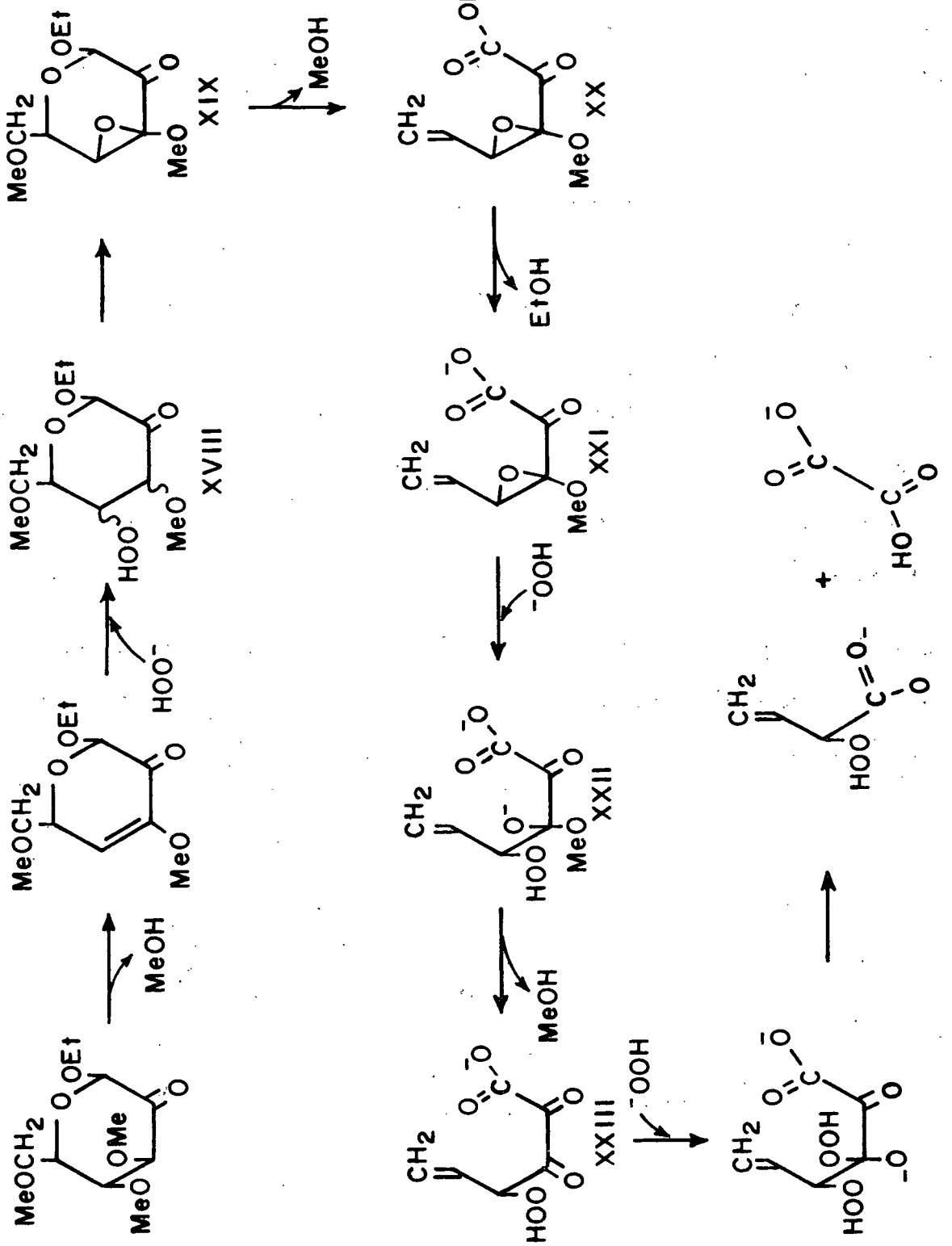


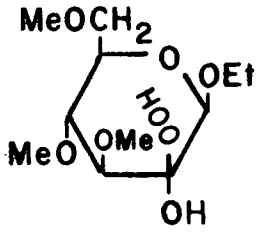
Figure 29. Postulated Mechanism Displaying the Effect that Hydrogen Peroxide has on the Alkaline Degradation Reaction

should result in higher levels of organic peroxides than were detected in either of these reactions (refer to Fig. 23 and 24). This discrepancy can be rationalized in two ways. Either the peroxidic product XXIV is not as stable as expected or the organic peroxide is not quantitatively hydrolyzed to hydrogen peroxide in the peroxide test samples.

ALKALINE PEROXIDE DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-GLUCOPYRANOSIDE

Ethyl 3,4,6-tri-O-methyl-β-D-glucopyranoside (ET3MG) underwent very slow degradation in 1.25N sodium hydroxide and 0.2M hydrogen peroxide at 60°C (Fig. 30). As the concentration of hydrogen peroxide slowly decreased, a significantly high amount of an intermediate organic peroxide was formed at early reaction time. This type of organic peroxide formation has been observed by other workers (1,2,22).

Weaver (1) suggested that the intermediate organic peroxide he measured was an α-hydroxyhydroperoxide. If that is the case, in this reaction it would be the α-hydroxyhydroperoxide of ET3M-KG shown below.



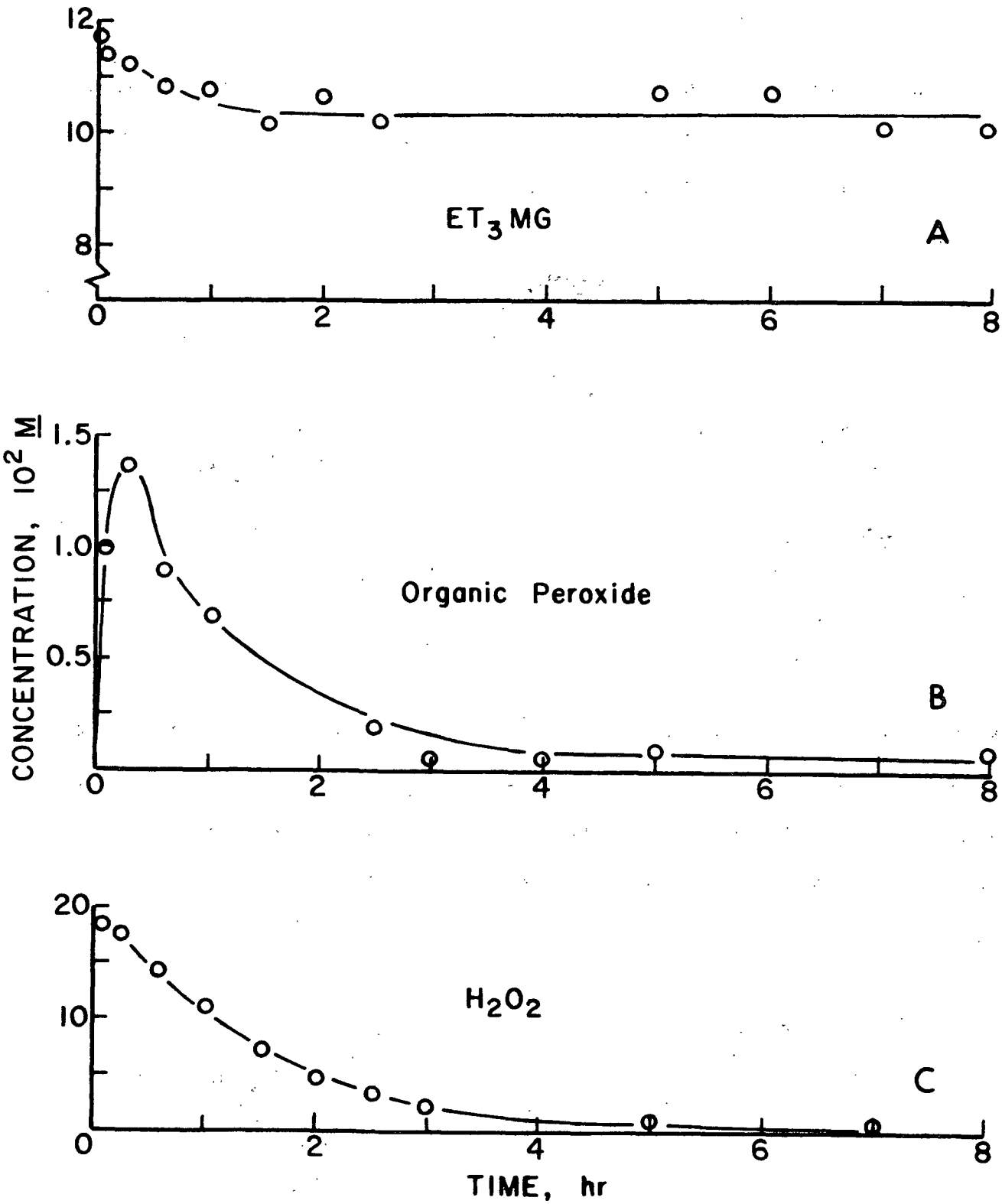
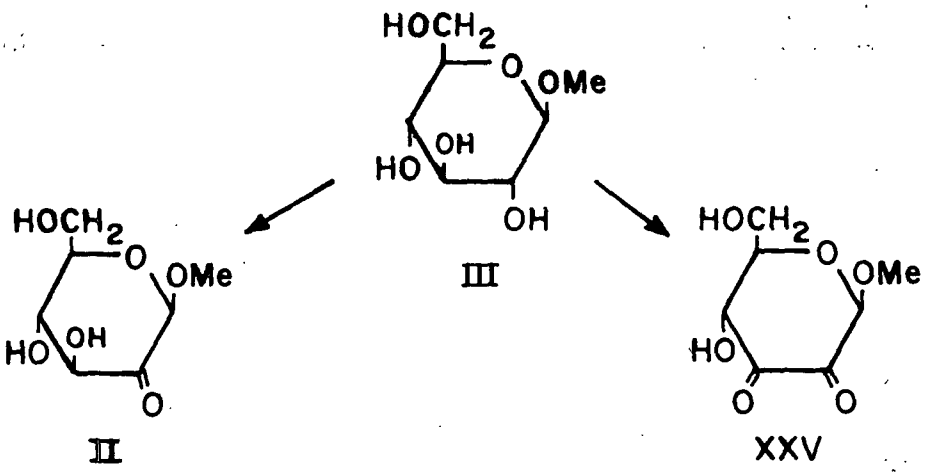


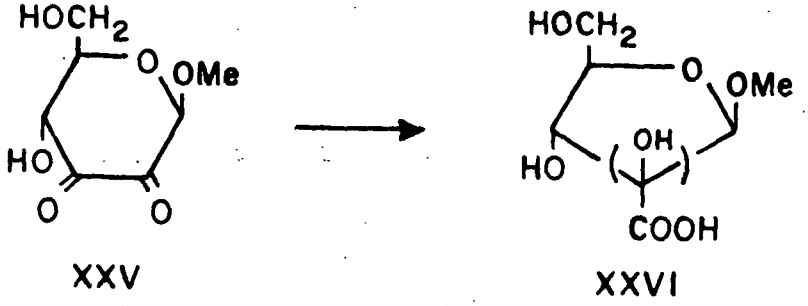
Figure 30. Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-glycopyranoside (A), Formation of an Organic Peroxide (B), and Consumption of Hydrogen Peroxide (C) in 1.25N NaOH at 60°C

IMPLICATIONS OF THIS WORK ON THE ALKALINE OXIDATIVE DEGRADATION OF GLYCOSIDES

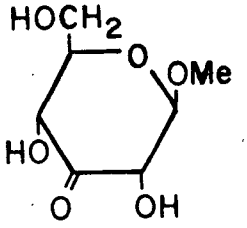
Ericsson (30) reported that the initial degradative step in the alkaline oxygen degradation of methyl β-D-glucopyranoside was either the glycosidulose (II) or the glucosid-2,3-diulose (XXV). Ericsson realized that in alkaline



solution keto-glycosides would rapidly undergo elimination reactions. Therefore, he postulated that the two keto groups were introduced into the glycosidic ring simultaneously (30). Postulation of intermediate XXV was necessary to explain the formation of the epimeric pair of branched acids (XXVI).

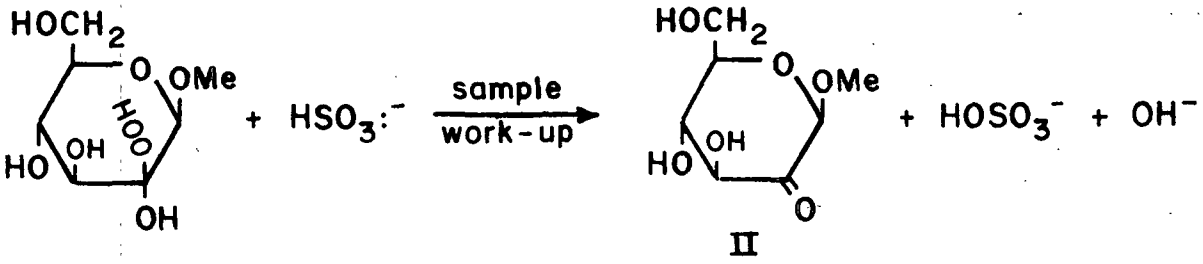


Yet, when Ericsson (30) reacted methyl β-D-ribo-hexopyranosid-3-ulose (V) in an alkaline oxygen solution, analysis of the reaction product mixture showed the two epimeric branched acids, XXVI, as major products. In order to form these acids, the keto-glycoside must have been stabilized with respect to the rapid alkaline elimination reaction.



I

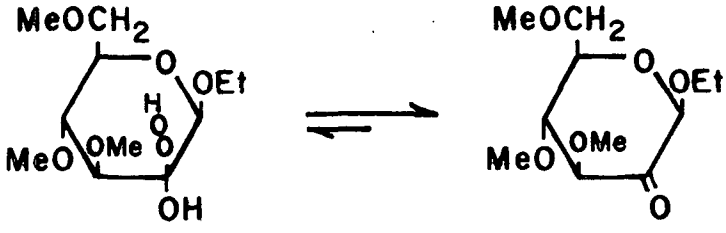
Weaver (1), in his work on the alkaline peroxide degradation of methyl β-D-glucopyranoside and methyl 4-O-methyl-β-D-glucopyranoside, postulated that nucleophilic addition of the hydroperoxide anion stabilized the keto-glycoside from elimination reactions. He based this postulation on the analysis of his reaction product mixtures. In this analysis, Weaver identified methyl β-D-arabino-hexopyranosidulose (II) from an early reaction sample (1). Realizing that Theander (23) had shown that this keto-glycoside was very reactive in alkaline solutions, Weaver proposed that during his sample work-up procedure, he had inadvertently formed the keto-glycoside (II) by reduction of an α-hydroxy-hydroperoxide intermediate. Weaver subsequently presented a reaction mechanism which presented the α-hydroxyhydroperoxide as the initial degradative intermediate in the alkaline peroxide degradation of methyl β-D-glucopyranoside and methyl 4-O-methyl-β-D-glucopyranoside.



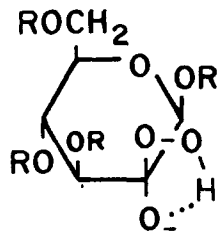
II

Millard (2) and Hearne (22) have also postulated α-hydroxyhydroperoxides as intermediates in the degradation of 1,5-anhydropentitols and the corresponding methyl pentosides in alkaline oxygen reactions. Furthermore they speculated that these hydroperoxides can rearrange to form branched acids analogous to XXVI.

In this work ET3MG was degraded under alkaline peroxide conditions. Although degradation was quite slow, an intermediate organic peroxide was formed in significant concentrations. But when attempts were made to deliberately form this organic peroxide from ET3M-KG, only insignificant amounts of organic peroxides could be measured. This suggests that although the keto-glycoside may be in equilibrium with the α-hydroxyhydroperoxide as Weaver (1) and others (2,22) have reported, the equilibrium is far toward the keto-glycoside.



The alkaline peroxide reaction of ET3MG supports the postulations of Weaver (1) and others (2,22), that the α-hydroxyhydroperoxide is the first formed intermediate in the alkaline oxidative degradation mechanism of glycosides. Even though an equilibrium may exist between the α-hydroxyhydroperoxide and the keto-glycoside and may favor the keto-glycoside, the α-hydroxyhydroperoxide can stabilize itself in alkaline solution by forming an intramolecular hydrogen bond (2) as shown below. This stabilization can be used to explain both the



relatively large amounts of organic peroxides observed in the alkaline peroxide degradation of ET3MG and also the stabilizing effect that the α-hydroxyhydroperoxide provides to the keto-glycoside that Weaver detected.

CONCLUSIONS

A mechanism has been proposed for the alkaline degradation of ethyl 3,4,6-tri-O-methyl-β-D-arabino-hexopyranosidulose (ET3M-KG). The keto-glycoside undergoes rapid degradation via a base-catalyzed β-elimination reaction to yield ethyl 4-deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose (α,β-KG) as its initial degradation intermediate. Subsequently, α,β-KG degrades to form several methoxy-containing products.

The initial step in the alkaline degradation of ET3M-KG which leads to α,β-KG is not affected by the addition of hydrogen peroxide to the alkaline reaction system. The subsequent degradation of α,β-KG in this system is postulated to proceed via an alkaline peroxide epoxidation mechanism which is most consistent with all of the experimental data that was gathered.

Ethyl 3,4,6-tri-O-methyl-β-D-glucopyranoside (ET3MG) was also degraded in alkaline hydrogen peroxide solution. The initial product of this degradation was an organic peroxide, presumably an α-hydroxyhydroperoxide. Previous workers (1,2,6,7,22) have hypothesized that the alkaline oxidative degradation of glycosides and anhydroalditols proceeds via an α-hydroxyhydroperoxide intermediate. The present study supports this hypothesis.

EXPERIMENTAL

ANALYTICAL METHODS

Melting points were determined on a Thomas Hoover Capillary melting point apparatus calibrated with known compounds.

Polarimetric measurements were made on a Perkin-Elmer 141 MC polarimeter equipped with a Honeywell Electronic 16 recorder.

Ultraviolet and visible spectroscopy were performed on a Cary 15 recording spectrophotometer.

Infrared spectra were recorded on Perkin-Elmer 700 and 621 grating spectrophotometers.

Proton magnetic resonance spectra were taken with a Varian A-60A spectrometer at ambient probe temperatures using tetramethylsilane as the internal standard. Nuclear magnetic resonance (both ^{1}H-NMR and ^{13}C-NMR) spectra were also taken with a Jeol FX-100 spectrometer equipped with a Texas Instrument 980 B computer.

Elemental analyses were performed by Chemalytics, Inc., Tempe, Arizona.

Thin-layer chromatography (TLC) used to follow the progress of synthetic reactions was conducted on microscope slides coated with silica gel G using methanolic sulfuric acid (5:1, wt.) spray with subsequent charring for spot detection. Solvent systems used for chromatographic separations were, (A) chloroform:ethyl acetate, 2:1 (vol.); (B) chloroform:ethyl acetate; 1:1 (vol.) (C) ethyl ether:light petroleum ether, 1:2 (vol.); and (D) ethyl acetate:light petroleum ether, 2:1 (vol.). Preparative TLC was performed on glass-backed

chromatoplates (5 x 20 cm) coated with silica gel GF (1 mm thickness).

Quantitative GLC was performed on Varian Aerograph 1200 and 2700 instruments equipped with hydrogen flame ionization detectors and recorders with Disc integrators. Preparative gas-liquid chromatography (GLC) was performed on a Varian Aerograph 712 chromatograph. Chromatographic conditions are listed in Appendix I.

SOLUTIONS AND REAGENTS

Triply-distilled water (44), anhydrous ethanol (45), anhydrous chloroform (46), anhydrous dichloromethane (46), and anhydrous dimethyl sulfoxide (47) were prepared by published procedures. Analytical grade reagents and solvents were used in all synthetic steps except when noted otherwise.

SODIUM HYDROXIDE STOCK SOLUTION

In order to reduce contamination by traces of heavy metal ions, the stock solution of sodium hydroxide was treated with a strong complexing agent. The procedure used for this purification process was adapted from the one described by Reiner and Poe (48). All of the glassware used in this procedure was first cleaned by soaking it in 35% (vol.) nitric acid followed by several rinses with triply-distilled water.

Sodium hydroxide (650 g) was dissolved in triply-distilled water (1700 mL boiled for 2 hr to remove dissolved carbon dioxide) in a 3-liter round-bottom flask. The complexing agent, phenyl 2-pyridyl ketoxime (1.2 g) was dissolved in a minimum of hot ethanol and added to the sodium hydroxide solution. A catalytic amount of 10% palladium on charcoal (0.2 g) was wetted with triply-distilled water and washed into the round-bottom flask. The mixture was heated to ca. 100°C

and stirred with a Teflon coated magnetic stirring bar. A stream of hydrogen gas was bubbled through the heated mixture for 8 hours, at which time the solution was stoppered and cooled overnight. The cooled mixture was filtered through a medium porosity sintered glass filter, and each liter of filtrate was immediately extracted with ethanol-isopentyl alcohol (1:3, vol.) (3 x 50 mL). The alcohol phase was deep red indicating that a complex between the phenyl 2-pyridyl ketoxime and ferrous ion had been formed (48).

The aqueous layer was returned to the cleaned 3-liter round-bottom flask and more complexing agent (1.2 g dissolved in a minimum of hot ethanol) was added. The solution was heated to ca. 100°C, and a stream of nitrogen gas was passed through the hot solution. After 12 hours, the deep wine red solution was cooled overnight. The solution was extracted again with the ethanol-isopentyl alcohol mixture (3 x 50 mL) and subsequently with chloroform (3 x 50 mL). The aqueous phase was heated to ca. 100°C and the hot solution was purged with nitrogen for 6 hours to remove residual organic solvents. After cooling to room temperature, the stock solution of sodium hydroxide was stored in a paraffin-lined, Teflon-stoppered glass bottle under a nitrogen atmosphere. GLC analysis demonstrated that there was no ethanol remaining in this solution. Comparison of the UV spectrum of the extracted solution to the spectrum of a solution that was not treated assured that all of the phenyl 2-pyridyl ketoxime had been effectively removed by extraction.

HYDROGEN PEROXIDE STOCK SOLUTION

Hydrogen peroxide (ca. 30%) was used as obtained from the manufacturer. The hydrogen peroxide solution was stored under refrigeration in a polyethylene bottle wrapped with aluminum foil. The strength of this stock solution, measured prior to each reaction, did not change appreciably over 2 years.

TITANIUM SULFATE REAGENT

The titanium sulfate reagent used for peroxide analysis was prepared as described by Weaver (1). Titanium sulfate (50 g, $TiSO_4 \cdot H_2SO_4 8H_2O$) was dissolved in concentrated sulfuric acid (50 mL) and diluted to 500 mL with triply-distilled water. After standing at room temperature for one week with intermittent shaking, the mixture was filtered through a medium porosity sintered glass filter and stored in a glass-stoppered bottle.

SODIUM THIOSULFATE SOLUTION

Anhydrous sodium thiosulfate (31.6 g) and sodium carbonate (0.2 g) were dissolved in freshly boiled, triply-distilled water (2 liters). The solution was standardized against potassium iodate solution to a starch end point.

PALLADIUM ON CHARCOAL CATALYST (10%)

The method of Monzingo (49) was used to prepare the catalyst. The catalyst (60.8 g) was powdered with a mortar and pestle and stored in tightly sealed bottles over calcium chloride.

2,4-DINITROPHENYLHYDRAZINE REAGENT

The 2,4-dinitrophenylhydrazine (2,4-DNP) reagent was prepared as described by Swinehart (50). 2,4-DNP (1.2 g) was dissolved in concentrated sulfuric acid (6 mL) and diluted with water (10 mL). While the solution was hot, 95% ethanol (32 mL) was added slowly so that none of the 2,4-DNP precipitated from the solution.

INTERNAL STANDARDS

Cyclohexyl 3,4,6-tri-O-methyl-β-D-glucopyranoside (CT3MG) was obtained from Dr. L. R. Schroeder as a chromatographically pure oil. CT3MG was used as

the internal standard in the kinetic analysis of the ET3M-KG alkaline and alkaline peroxide reactions.

Reagent grade n-propyl alcohol was used as the internal standard in the alcohol analyses of all reactions.

ETHYL 4,6-DI-O-METHYL-β-D-GLUCOPYRANOSIDE (XXVII)

XXVII was used as the internal standard in the GLC product analyses. The synthetic steps involved in its preparation are described below.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl Bromide (XXVIII)

The following procedure has been adapted from Bates (51). Penta-O-acetyl-α,β-D-glucopyranose (200 g) was treated with hydrogen bromide in glacial acetic acid [32-33% (wt.), 100 mL]. After 2 hours at room temperature, chloroform (800 mL) was added and the solution was poured into ice water (3 liters) under rapid stirring. The chloroform layer was separated and washed with ice water (2 x 2000 mL), dried with calcium chloride, filtered and concentrated in vacuo until crystallization started. The sirup was dissolved in absolute ether (500 mL), and crystallization occurred at room temperature with the addition of light petroleum ether. Yield: 185.3 g (87.9%), m.p. 87-88°C literature (51) 88-89°C.

Ethyl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside (XXIX)

A modified Koenings-Knorr procedure (52) was used in this preparation. Drierite (10-20 mesh, 207 g), yellow mercuric oxide (70.0 g), mercuric bromide (5.2 g), chloroform (1.5 liters), and anhydrous ethanol (500 mL) were vigorously stirred in a 3-necked Morton flask for 0.5 hour. XXVIII (140 g) was added and after ca. 8 hours at room temperature the reaction was complete (TLC, solvent

A). The mixture was filtered through Celite and the filtrate was washed with 20% aqueous potassium iodide (2 x 21) and water (2 liters). The organic layer was concentrated *in vacuo* to dryness (120.3 g). The dried crystalline residue was dissolved in hot ethanol (400 mL) and allowed to crystallize overnight in the refrigerator. Yield: 110.5 g (86.2%), m.p. 105-106°C, $[\alpha]_D$ -22.1° (c, 0.983, $CHCl_3$); literature (52): m.p. 106-107°C, $[\alpha]_D$ -22.3° ($CHCl_3$).

Ethyl β-D-Glucopyranoside (XXX)

XXIX (260.4 g) was dissolved in hot methanol (2 liters) and a catalytic amount of sodium methoxide (20 mL of 0.1N solution) was added to the solution. After 20 minutes reflux the reaction was complete (TLC, solvent A). The solution was deionized (IR-120), decolorized with charcoal and concentrated *in vacuo* to a thick sirup. The sirup was dissolved in hot ethyl acetate (5 liters). Crystallization proceeded very slowly at room temperature. Yield: 117.1 g (81.4%), m.p. 82-83.5°C, $[\alpha]_D$ -39.2° (c, 1.021, H_2O); literature (53): m.p. 90.4°C, $[\alpha]_D$ -36.7° (H_2O).

Ethyl 4,6-*O*-Benzylidene-β-D-glucopyranoside (XXXI)

Compound XXX (64.2 g) was dissolved in benzaldehyde (250 mL). Powdered anhydrous zinc chloride (125 g) was added and the resulting mixture was stirred vigorously in a sealed flask at room temperature for 4 days. The product was poured into a mixture of ice and 10% aqueous sodium bisulfite (2 liters) and stirred vigorously for one hour. The crystalline material was filtered and washed with cold 10% aqueous sodium bisulfite (2 x 1 liter), cold saturated aqueous sodium bicarbonate (2 x 500 mL), cold water (2 x 500 mL) and cold light petroleum ether (500 mL). The wash-water was saved, as it contained much of the product. The yield of crystalline material at this point was 44.1 g.

The aqueous wash solutions were combined, neutralized to pH 7-8 with sodium bicarbonate, and extracted with chloroform until no product remained in the aqueous phase (TLC, solvent B). The chloroform extracts were combined and concentrated *in vacuo* to dryness. The crystalline residue was washed thoroughly with cold light petroleum ether to remove trace amounts of benzaldehyde. The yield of this step was 21.0 g. Total yield: 65.1 g (71.3%), m.p. 173-173.5°C, $[\alpha]_D$ -74.3° (c, 0.970, DMF), $[\alpha]_D$ -60.5° (c, 1.136, $CHCl_3$). (Found: C, 60.6%; H, 7.0%. $C_{15}H_{20}O_6$ requires C, 60.8%; H, 6.8%.) The ^{1}H-NMR spectra of XXXI in DMSO-d_6 showed *inter alia*: a 5-proton multiplet at δ 7.20-7.55 indicative of a single phenyl moiety; a one proton singlet at δ 5.55 indicative of the non-aromatic benzylidene proton; and a 3 proton triplet at δ 0.97-1.34 assigned to the methyl group of the aglycone.

Ethyl 2,3-di-*O*-Benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (XXXII)

The following procedure was adapted from the one described by Brimacombe, *et al.* (54). Compound XXXI (32.6 g) was dissolved in dimethylformamide (100 mL). Sodium hydride (19.1 g) was slowly added to the solution and the mixture was stirred vigorously with the exclusion of moisture overnight at room temperature. Benzyl chloride (2 x 50 mL) was added to the reaction mixture. Upon completion of the reaction (TLC, solvent C), excess sodium hydride was destroyed by careful addition of methanol. After the effervescence ceased, the solution was concentrated *in vacuo* to dryness. The crystalline residue was partitioned between water (400 mL) and chloroform (1000 mL). The organic phase was washed with water (3 x 250 mL); each water wash was twice back-extracted with chloroform (2 x 50 mL). The combined chloroform phases were dried (Na_2SO_4), filtered and concentrated *in vacuo* to dryness. The product crystallized spontaneously from acetone by addition of water. Yield: 46.1 g (86.3%), m.p. 115-116°C, $[\alpha]_D$ -40.2°C (c, 1.00, $CHCl_3$). (Found: C, 72.9%; H, 6.8%. $C_{29}H_{32}O_6$ requires

C, 73.1%; H, 6.7%.) The ^{1}H-NMR spectra of XXXII in $CDCl_3$ showed inter alia: a 15 proton multiplet at δ 7.12-7.58, indicative of the three phenyl moieties (i.e., the benzylidene plus the two benzyl substituents); a one proton singlet at δ 5.54 assigned to the nonaromatic benzylidene proton; a 4 proton singlet at δ 4.87 attributed to the four benzyl protons; and a three proton triplet at δ 1.13-1.44 assigned to the methyl group in the aglycone.

Ethyl 2,3-di-O-Benzyl-β-D-glucopyranoside (XXXIII)

The procedure of Bell and Lorber (55) was used to remove the benzylidine substituent. Compound XXXII (18.9 g) was dissolved in acetone (250 mL), water (50 mL), and 3N HCl (50 mL). The solution was refluxed until TLC (solvent B) showed that the reaction was complete, usually 5-8 hours. The solution was cooled, neutralized to ca. pH 8 with saturated sodium bicarbonate solution, and concentrated in vacuo to dryness. The residue was extracted with hot chloroform (500 mL), filtered to remove inorganic salts and concentrated in vacuo to a sirup. The product crystallized from ethyl acetate by addition of light petroleum ether. Yield: 12.9 g (83.9%), m.p. 76-76.5°C, $[\alpha]_D$ -23.9° (c, 0.991, $CHCl_3$). (Found: C, 67.8%; H, 7.1%. $C_{22}H_{28}O_6$ requires C, 68.0%; H, 7.2%.) The ^{1}H-NMR spectra ($CDCl_3$) showed inter alia: a 10 proton multiplet at δ 7.12-7.58 indicative of the two phenyl moieties; a pair of two proton singlets at δ 4.76 and 4.99 assigned to the benzyl protons; and a 3 proton triplet at δ 1.15-1.40, attributed to the methyl group of the aglycone.

Ethyl 2,3-di-O-Benzyl-4,6-di-O-methyl-β-D-glucopyranoside (XXXIV)

Compound XXXIII (72.6 g) was dissolved in tetrahydrofuran (THF, 946 mL). Powdered sodium hydroxide (170 g) was added and the mixture was stirred for 30 minutes in a 3-liter Morton flask. Dimethyl sulfate (120 mL) was added dropwise over 8 hours to the vigorously stirred mixture. After an additional 16

hours, TLC (solvent B) showed that the reaction was complete. Water (500 mL) was added and the temperature of the reaction mixture was raised to 65°C to destroy residual dimethyl sulfate. After 0.5 hour at 65°C, dry air was passed over the stirred solution for an additional 2 hours to remove most of the THF. It was necessary to remove as much of the THF as possible to prevent emulsions from occurring later in the washing steps. The aqueous solution was cooled to room temperature and extracted with chloroform (2 x 500 mL, 1 x 200 mL). Each chloroform extract was washed with water (1 x 200 mL), combined and concentrated in vacuo to a yellow oil (78.1 g, 100%). A portion of the sirup was purified by column chromatography (2.5 x 100 cm column packed with silica gel 60-200 mesh; solvent ethyl acetate-chloroform, 1:20 vol.) for characterization: $[\alpha]_D$ +23.2° (c, 2.613, $CHCl_3$). (Found: C, 65.7%; H, 7.5%. $C_{24}H_{32}O_6$ requires C, 69.2%; H, 7.7%.) The ^{1}H-NMR spectrum run in $CDCl_3$ showed inter alia: a 10 proton multiplet at δ 6.91-7.66, caused by the two phenyl moieties from the benzyl groups; two 3 proton singlets at δ 3.40 and 3.52, assigned to the two methyl ether groups; and a 3 proton triplet at δ 1.10-1.44, attributed to the methyl group in the aglycone.

Ethyl 4,6-di-O-Methyl-β-D-glucopyranoside (XXXV)

The following procedure was adapted from Ekborg, et al. (56). Compound XXXIV (15.0 g) was dissolved in absolute ethanol (150 mL), and the solution was placed in a Parr hydrogenation bomb. Ten percent palladium on charcoal catalyst (1.5 g) was added to the solution, and the bomb was sealed, purged with hydrogen gas, and pressurized at 40 psig with hydrogen gas. After 48 hours of vigorous stirring at room temperature, TLC (solvent C) showed that the reaction was complete. The spent catalyst was recovered by filtering the solution through a Celite pad. The pad was washed with acetone (2 x 100 mL), and the filtrate was

concentrated in vacuo to dryness. The crystalline residue (11.3 g) was crystallized from isopropyl ether. Characterization: m.p. 77-78°C, $[\alpha]_D$ -30.9° (c, 0.999, $CHCl_3$). (Found: C, 50.7%; H, 8.3%. $C_{10}H_{20}O_6$ requires C, 50.8%; H, 8.5%.) The ^{1}H-NMR spectrum ($CDCl_3$) showed inter alia: a one proton doublet at δ 4.19-4.27 attributed to the anomeric proton; two 3 proton singlets at δ 3.40 and 3.58, assigned to the methoxy groups; and a 3 proton triplet at δ 1.09-1.36 assigned to the methyl group of the aglycone.

PREPARATION OF MODEL COMPOUNDS

ETHYL 3,4,6-TRI-O-METHYL-β-D-arabino-HEXOPYRANOSIDULOSE (ET3M-KG)

3,4,6-Tri-O-acetyl-1,2-O-(1-exo-ethoxyethylidene)-α-D-glucopyranose (XXXVI)

This procedure is a modification of the one developed by Hultman, et al. (34). Glucosyl bromide (XXVI) (187.6 g), tetraethylammonium bromide (158 g), lutidene (50 g) and anhydrous ethanol (50 g) was dissolved in anhydrous chloroform (1500 mL) and the solution was refluxed for 1.5 hours with the exclusion of moisture. After cooling to 0°C, the chloroform solution was washed with ice water (3 x 1000 mL), dried over sodium sulfate, filtered and concentrated in vacuo to a thick sirup. The crude product was crystallized from isopropyl ether containing a trace of pyridine. Yield: 115.5 g (67.3%), m.p. 93.5-96°C; literature (57): m.p. 95-96°C.

3,4,6-Tri-O-methyl-1,2-O-(1-exo-ethoxyethylidene)-α-D-glucopyranose (XXXVII)

The following procedure was adapted from the one outlined by Hultman, et al. (34). Compound XXXVI (115.5 g) was dissolved in THF (1892 mL) in a 3-liter Morton flask equipped with condenser, dropping funnel and an overhead stirrer.

Powdered sodium hydroxide (260 g) was added, and the slurry was vigorously stirred. Dimethyl sulfate (175 mL) was added dropwise over 4 hours. After an additional 3 hours, TLC (solvent B) showed that the reaction was complete. Benzene (950 mL), triethylamine (300 mL), and enough water to dissolve all solids were added. The bath temperature was raised to 65°C to destroy residual dimethyl sulfate, and dry air was passed over the reaction solution to remove most of the THF. After 2 hours the solution was cooled to 0°C and the stirrer was stopped. The organic phase was separated and washed with 1% aqueous potassium iodide (1000 mL), 1% aqueous sodium thiosulfate (1000 mL), and water (2 x 1000 mL). Each aqueous phase was back-extracted with benzene (600 mL). The benzene extracts were combined, dried (Na_2SO_4) and concentrated *in vacuo* to a thin yellow oil (84.5 g; 94.2%). The crude XXXVII was not characterized due to its lability.

Ethyl 3,4,6-Tri-*O*-methyl-β-D-glucopyranoside (ET3MG)

Crude XXXVII (84 g) was dissolved in anhydrous ethanol (500 mL) containing concentrated sulfuric acid (1.0 mL). After 12 hours at room temperature, TLC (solvent B) showed the major product to be ET3MG, with minor amounts of ethyl 2-*O*-acetyl-3,4,6-tri-*O*-methyl-β-D-glucopyranoside and hydrolysis products. The solution was diluted with 2*N* aqueous sodium hydroxide (200 mL) and refluxed for 3 hours, to deacetylate or degrade the minor products. After cooling to room temperature, the dark brown solution was neutralized to *ca.* pH 8 with sulfuric acid and concentrated *in vacuo*. The product was dissolved in hot $CHCl_3$ (750 mL), filtered to remove insoluble inorganic salts and concentrated *in vacuo* to a brown sirup. ET3MG was crystallized from the sirup in petroleum ether (b.p. 60-110°C). Yield: 27.0 g (37.3%). Recrystallization from petroleum ether (b.p. 30-60°C) yielded the pure product; m.p. 52-52.5°C, $[\alpha]_D$ -23.8° (c, 0.999, $CHCl_3$); literature (34): m.p. 52-53.5°C, $[\alpha]_D$ -23.8° ($CHCl_3$).

Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (ET3M-KG)

ET3MG (10.0 g) was dissolved in anhydrous DMSO (40 mL). Acetic anhydride (7 mL) was added, and the flask was sealed under a nitrogen atmosphere. The solution was stirred at room temperature for 24 hours. After the reaction solution was freeze-dried, the crude product was crystallized from light petroleum ether and sublimed three times to ensure purity (sublimation conditions: bath temperature 60-63°C; vacuum, 0.01-0.05 mm Hg). Yield: 4.7 g (47.4%), m.p. 61.5-62°C, $[\alpha]_D$ -88.6° (c, 1.33, $CHCl_3$), $[\alpha]_D^{25°}$ -35.0° (c, 1.046, H_2O), $[\alpha]_{546.1}^{25°}$ -41.4° (c, 1.046, H_2O) (Found: C, 53.2%; H, 8.0%. $C_{11}H_{20}O_6$ requires C, 53.2%; H, 8.0%.) The ^{1}H-NMR spectrum ($CDCl_3$) showed inter alia: a one proton singlet at δ 4.79, assigned to the anomeric proton; three 3 proton singlets at δ 3.42, 3.54 and 3.58 attributed to the methyl ether substituents; and a 3 proton triplet at δ 1.12-1.40 assigned to the methyl group in the aglycone. ^{13}C-NMR spectrum ($CDCl_3$) showed: C-2, 156.83 ppm; C-1, 98.82 ppm; C-3, 87.83 ppm; C-4, 81.80 ppm; C-6, 71.49 ppm; CH_2 of the ethyl aglycone, 65.12 ppm; OMe, 60.26, 59.43, and 59.33 ppm; and CH_3 of the ethyl aglycone, 14.93 ppm. The IR spectrum showed a strong carbonyl stretch at 1750 cm^{-1}. The UV spectrum showed $\lambda_{max}^{H_2O}$ 255 nm, ε ca. 10-20. ^{1}H-NMR, ^{13}C-NMR, and IR spectra as given in Appendix V.

A small portion of the product (110 mg) was reduced with sodium borohydride (3 x 400 mg) in methanol (30 mL) and DMF (2 mL). The solution was stirred until effervescence ceased and concentrated in vacuo to a sirupy residue which was dissolved in water (50 mL) and extracted with chloroform (4 x 50 mL). The chloroform extracts were washed with water until the water was neutral. The chloroform was dried (Na_2SO_4) and concentrated in vacuo to a sirup (170 mg). Gas chromatographic analysis showed that only two products were formed in this reduction. The major product (ca. 98%) had a retention time identical to a

known sample of ethyl 3,4,6-tri-O-methyl-β-D-mannopyranoside (ET3MM). The other product (<2%) had the same retention time as ET3MG. The sirup was crystallized from light petroleum ether. Yield: 45 mg, m.p. 62.5-63°C, $[\alpha]_D^{19°}$ -77.0° (c, 0.91, $CHCl_3$); literature (58): (ET3MM) m.p. 62.5-64°C, $[\alpha]_D^{27°}$ -79.6° ($CHCl_3$).

Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose 2,4-Dinitrophenylhydrozone

Three drops of the ET3M-KG crude oxidation mixture in a chloroform solution were added to 2,4-dinitrophenylhydrazine reagent (2 mL). Orange crystals were formed immediately. The solution was held at ca. 4°C for two hours. The product was collected and recrystallized from ethanol-water; m.p. 233-234°C, $[\alpha]_D$ +233.1° (c, 0.16, $CHCl_3$).

ETHYL 4-DEOXY-3,6-DI-O-METHYL-β-D-glycero-HEX-3-ENOPYRANOSIDULOSE (α,β-KG)

α,β-KG was prepared from ET3M-KG by two methods. The first method involved a controlled base-catalyzed elimination reaction, and the second method involved reaction on a silica gel support.

Method I

This procedure was adapted from one described by Svensson (24). ET3M-KG (3.9 g) was dissolved in anhydrous dichloromethane (75 mL) and treated with 0.1N sodium n-propoxide in n-propanol (75 mL). The reaction was monitored with TLC (solvent D). All of the ET3M-KG was converted to α,β-KG in less than 5 minutes. The reaction was neutralized with 4M aqueous acetic acid and concentrated in vacuo to dryness (bath temperature less than 40°C). The crude product was purified by column chromatography (2.5 x 100 cm column packed with silica gel 60-200 mesh; solvent D). After several column purifications α,β-KG was

obtained as a pure liquid (2.7 g). Distillation through a short path Kontes vacuum distillation apparatus (pressure, 0.05-0.03 mm Hg; head temperature, 85-87°C, and pot temperature 110-115°C) yielded α,β-KG as a chromatographically pure, colorless sirup; yield: 1.2 g (35.3%).

Method II

Mother liquor (9.4 g) from the crystallization of ET3M-KG (TLC showed that much ET3M-KG remained in these mother liquors) was passed over a column of activated silica gel. (The silica gel was treated at 110°C for 20 hours prior to loading the column.) The fractions that contained α,β-KG were located, isolated and concentrated *in vacuo* to a yellow sirup. This sirup was distilled through a Kontes short path vacuum distillation apparatus (pressure, 0.03 mm Hg, head temperature, 85°C, pot temperature, 104-106°C) to obtain α,β-KG as a colorless sirup that was chromatographically pure. Yield: 2.29 g.

Characterization: $[\alpha]_D^{25^\circ}$ -89.4°, $[\alpha]_{546.1}^{25^\circ}$ -105.9° (c, 0.596, $CHCl_3$); $[\alpha]_D^{25^\circ}$ -59.2, $[\alpha]_{546.1}^{25^\circ}$ -70.5° (c, 1.368, H_2O). The ^{1}H-NMR spectrum showed *inter alia*: a one proton doublet at δ 5.90-5.94, attributed to the olefinic proton on C-4; a one proton singlet at δ 4.94, assigned to the anomeric proton; singlets for two methoxyl groups at δ 3.44 and 3.67; and a sextet at δ 4.85-4.95 assigned to C-5. The ^{13}C-NMR spectrum ($CHCl_3$) indicated: C-2, 184.45 ppm; C-3, 147.77 ppm; C-4, 114.08 ppm; C-1, 98.49 ppm; C-5, 75.35 ppm; C-6, 71.60 ppm; CH_2 of the ethyl aglycone, 64.80 ppm; OMe, 59.06 and 54.90 ppm; and CH_3 of the ethyl aglycone, 15.00 ppm. The IR spectrum indicated *inter alia*: no OH stretch, strong C-H stretch at 2850-2950 cm^{-1} (saturated C-H) and weak at 3050 cm^{-1} (unsaturated C-H); very strong bands at 1710 cm^{-1} (carbonyl) and 1640 (conjugated C=C), strong broad bands near 1100 cm^{-1} (C-O-C bending vibrations). The UV spectrum shows a single absorption maximum at 263 nm (ε_{H_2O} 4,400). Elemental

analysis of this compound could not be obtained due to its lability. ^{1}H-NMR, ^{13}C-NMR, UV, and IR spectra are given in Appendix V.

REACTION ANALYSIS

CONDITIONING OF REACTION GLASSWARE

Before each reaction, all glassware used in the preparation and sampling of the reaction solution was passivated to prevent surface catalyzed decomposition of hydrogen peroxide (62). The procedure used to passivate the glassware required overnight soaking in: Alconox, 10% sodium hydroxide, 35% nitric acid, and 30% hydrogen peroxide, followed by thorough rinsing with distilled water and finally triply-distilled water. The glassware was dried at 110°C and stored in a desiccator over sodium hydroxide pellets.

PREPARATION OF REACTION SOLUTIONS

Keto-glycoside Reactions

The keto-glycoside was weighed into a passivated volumetric flask. Standardized sodium hydroxide stock solution was weighed into a larger passivated volumetric flask. Where applicable hydrogen peroxide stock solution was weighed into a passivated 50-mL beaker. Using triply-distilled water, the hydrogen peroxide was quantitatively washed into the volumetric flask containing sodium hydroxide. Finally triply-distilled water was used to dilute the alkaline solution to volume, and the solution was equilibrated at 25°C in a water bath.

To initiate the reaction, the alkaline or alkaline peroxide solution was used to dilute the keto-glycoside to reaction volume. The reaction solution was shaken vigorously to ensure thorough mixing and poured into a dry, passivated, 50-mL beaker thermostated at 25°C (±0.05°) in a water bath. Passivated

pH electrodes were placed directly into the reaction solution, and the pH of the reaction solution was recorded continuously throughout the reaction.

Ethyl 3,4,6-Tri-O-methyl-β-D-glucopyranoside Reaction

The reactor and valving system used in this reaction have been described by Hearne (22). Prior to use the reactor and valve systems were cleaned with the passivating solutions. In preparation for the reaction ET3MG was dissolved in an alkaline solution and placed in a Teflon-lined reactor which was sealed, purged with nitrogen, and equilibrated at 60°C in an oil bath. Stock hydrogen peroxide solution was thermostated at 60°C in a water-jacketed addition buret for 1.0 hour prior to reaction. To initiate the reaction the hydrogen peroxide solution was blown into the reactor with nitrogen pressure. The addition buret was quickly rinsed with enough triply-distilled water to bring the total reaction volume to 200 mL. Initial reaction concentrations were: ET3MG, 0.118M; hydrogen peroxide, 0.201M; and hydroxide, 1.250N.

Reactions Monitored by Ultraviolet Absorption

Stock aqueous solutions of sodium hydroxide and ET3M-KG or α,β-KG were prepared so that when aliquots of these solutions were diluted to 3.5 mL with triply-distilled water their concentrations would be 0.05N sodium hydroxide and 8×10^{-5}M keto-glycoside. The reactions were initiated in the UV cuvette by addition of the alkali to the diluted keto-glycoside solution, followed by thorough mixing. On the average it took less than 20 seconds to initiate the reactions. The average scanning time from 400 nm to 200 nm was 35 seconds. All absorbance measurements were made using triply-distilled water as the reference.

Reactions Monitored by Optical Rotation

All optical rotation measurements were made at 546.1 nm of the mercury spectrum using a passivated polarimetry cell.

The reaction solutions were prepared in a polarimetry cell by dilution of stock solutions that were thermostated at 25°C. Enough keto-glycoside stock solution was added so that a final concentration of 0.056M was obtained on dilution to reaction volume. The reactions were initiated by addition of the stock alkali solution to the cell; this addition brought the reaction volume to 4.5 mL. After all reaction components had been added to the cell, the filling ports were fitted with Teflon plugs, and the reaction solution was thoroughly mixed by shaking vigorously. The average time lag between reaction initiation and recording of optical rotation data was one minute.

GLYCOSIDE ANALYSIS

Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose Analysis

At the desired sampling time, a reaction sample (1 mL) was added to a flask containing accurately determined amounts of internal standards (CT3MG and n-PrOH) and an excess of sodium borohydride (ca. 200 mg). Each sample was allowed to reduce overnight at room temperature. A small portion (ca. 0.1 mL) of each sample solution was placed in a separate sample vial and cooled to ca. 2°C for alcohol analysis. Cation exchange resin (IR-120, H^+ ca. 5 mL) was added to the rest of the sample to quench the residual sodium borohydride. Each sample was deionized by passing it through MB-3 (H^+, OH^-) ion exchange resin (50 mL). The resin column was washed with water, and the eluates were concentrated in vacuo to dryness. Methanol (3 x 25 mL) was added and the solution concentrated in vacuo to a sirup after each addition. Samples were stored as chloroform

solutions at ca. 2°C until they could be analyzed by GLC in triplicate under conditions described in Appendix I. All concentrations were performed at bath temperatures of less than 40°C to reduce the chance of evaporating the relatively volatile ethyl tri-O-methyl glycosides.

Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose Analysis

Samples (0.1 mL) were taken from the reaction solution and immediately quenched with 4M acetic acid (0.05 mL). The solution was applied to a preparative TLC plate and developed with ethyl acetate. The α,β-KG was located on the chromatograms by its ability to absorb UV light emitted by a zinc phosphor in the silica gel. The α,β-KG-containing band was scraped from the plate into a fine-porosity sintered glass funnel. The α,β-KG was extracted from the silica gel with methanol (10 x 10 mL) and concentrated in vacuo to dryness. The crystalline residue (containing much $CaSO_4$ binder that was also extracted from the silica gel) was extracted with chloroform (5 x 10 mL), filtered and concentrated in vacuo to a sirup. All concentrations were performed at bath temperatures of less than 40°C. The sirup was diluted volumetrically with chloroform, and the absorbance was measured at 260 nm against a chloroform reference. The concentration of α,β-KG was obtained from a Beer-Lambert straight line calibration curve (Fig. 31). A control sample containing a known amount of α,β-KG in 4M acetic acid (0.05 mL) was analyzed using the same procedure described above. This control provided an estimation of the recovery efficiency from the silica gel. The efficiency was always from 97-100%. All samples were corrected for the efficiency of recovery from silica gel.

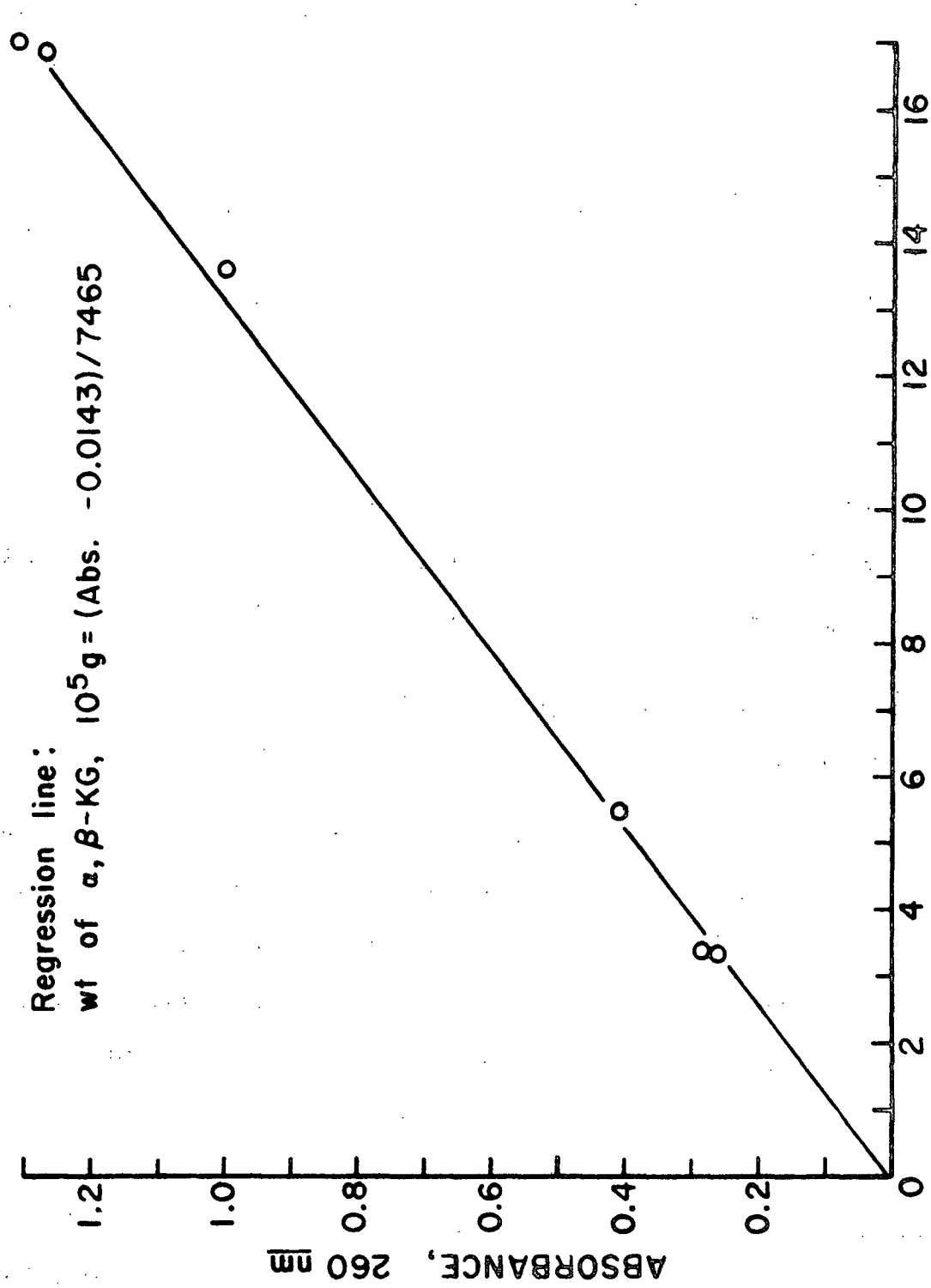


Figure 31. Beer-Lambert Calibration Curve for α,β-KG in Chloroform Solution

Ethyl 3,4,6-Tri-O-methyl-β-D-glucopyranoside Analysis

Samples (1 mL) were added to a flask containing accurately measured amounts of aqueous internal standard solutions (ET3MG and n-PrOH) and 1M $NaHSO_3$ (2 mL), which quenched the residual hydrogen peroxide. Each sample was deionized by passing it through MB-3 (H^+, OH^-) ion exchange resin and concentrated *in vacuo*. The samples were stored as chloroform solutions at *ca.* 2°C until they could be analyzed in triplicate by GLC under conditions described in Appendix I. All concentrations were performed at bath temperatures of less than 40°C.

ALCOHOL ANALYSIS

Samples used for glycoside analysis that had been reduced with sodium borohydride were analyzed directly by GLC in triplicate (59). Appendix I gives the chromatographic conditions for the analysis.

The stability of methanol and ethanol was demonstrated at reaction conditions more severe than those used in these reactions.

ET3M-KG and α,β-KG were thermally unstable and liberated methanol and ethanol on GLC analysis using injection port temperatures greater than 90°C. It was for this reason that the reduced samples were used for alcohol analyses. The products of the sodium borohydride reduction of α,β-KG and ET3M-KG released no methanol or ethanol at injection port temperatures below 120°C.

PEROXIDE ANALYSIS

Peroxide analysis involved a colorimetric method employing acidic titanium sulfate (41-43). Titanium(IV) cation immediately forms a stable colored complex with hydrogen peroxide. Therefore, the initial absorbance of samples treated

with titanium sulfate reagent was taken as a measure of hydrogen peroxide concentration. With time, most organic peroxides undergo acid hydrolysis to yield hydrogen peroxide which would become available to react with titanium(IV) cation. Reaction samples generally increased in absorbance with time, and the difference between the final absorbance and initial absorbance was attributed to the hydrolysis of organic peroxides. A straight line calibration curve was constructed by analyzing various solutions of hydrogen peroxide with a standard iodide-thiosulfate titration method (60) and the titanium sulfate colorimetric method (Appendix II).

A sample (0.5 mL) of the reaction solution was mixed with titanium sulfate reagent (1 mL) and enough sulfuric acid to give a final pH of 1 or 0 when the sample was diluted to 25 mL with distilled water. Two peroxide measurements (one at pH 1 and one at pH 0) were made at each sample time. Generally the pH 0 sample reached its maximum absorption before the pH 1 sample. The amount of sulfuric acid needed to lower the pH to the desired level was predetermined by using a pH meter. Absorption measurements were made at 400 nm within 5 minutes using a distilled water blank. The absorbance of each sample was measured over a period of 24 hours. When the absorbance of a sample increased with time, it seemed to reach maximum absorbance between 5 and 9 hours. Generally the difference between the maximum absorbance at pH 0 and the initial absorbance at pH 1 was used to determine the concentration of organic peroxide.

PRODUCT ANALYSIS

Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose Analysis

The concentration of α,β-KG was determined by two methods. Except where noted otherwise, the first method was used to determine the concentration to

α,β-KG in the alkaline degradations of ET3M-KG, while the second method was used for the alkaline peroxide degradations of ET3M-KG.

Method I

When α,β-KG was reduced with sodium borohydride, several products were formed as a result of 1,2- and 1,4-addition to the α,β-unsaturated conjugated system. These products happened to elute in two major areas [AB (1) and AB (2) in Fig. 32] on the gas chromatograms of the keto-glycoside analytical samples. These series of peaks were integrated, and a relative concentration profile was plotted. Since the reduction mixture was so complex, a detector response factor could not be generated. Therefore, the actual concentration of α,β-KG during the reaction of ET3M-KG was measured at certain points in the curve by a TLC-UV analysis already described in this report. This TLC-UV analysis was complicated in this case by the fact that residual ET3M-KG is partially converted to α,β-KG when it is developed on a silica gel plate. Therefore each sample was corrected for the conversion ratio of a known amount of ET3M-KG to α,β-KG. This ratio was 8-10% on the chromatoplates used for this analysis.

Alternatively, acid quenched samples from the ET3M-KG alkaline degradation reaction were analyzed by GLC (Appendix I for conditions). This method was calibrated by correction for difference in detector response between equivalent molar amounts of α,β-KG and the internal standard. These two methods agreed remarkably well.

Method II

In this method the concentration of α,β-KG in the alkaline peroxide reaction solution of ET3M-KG was measured directly by UV analysis. A reaction

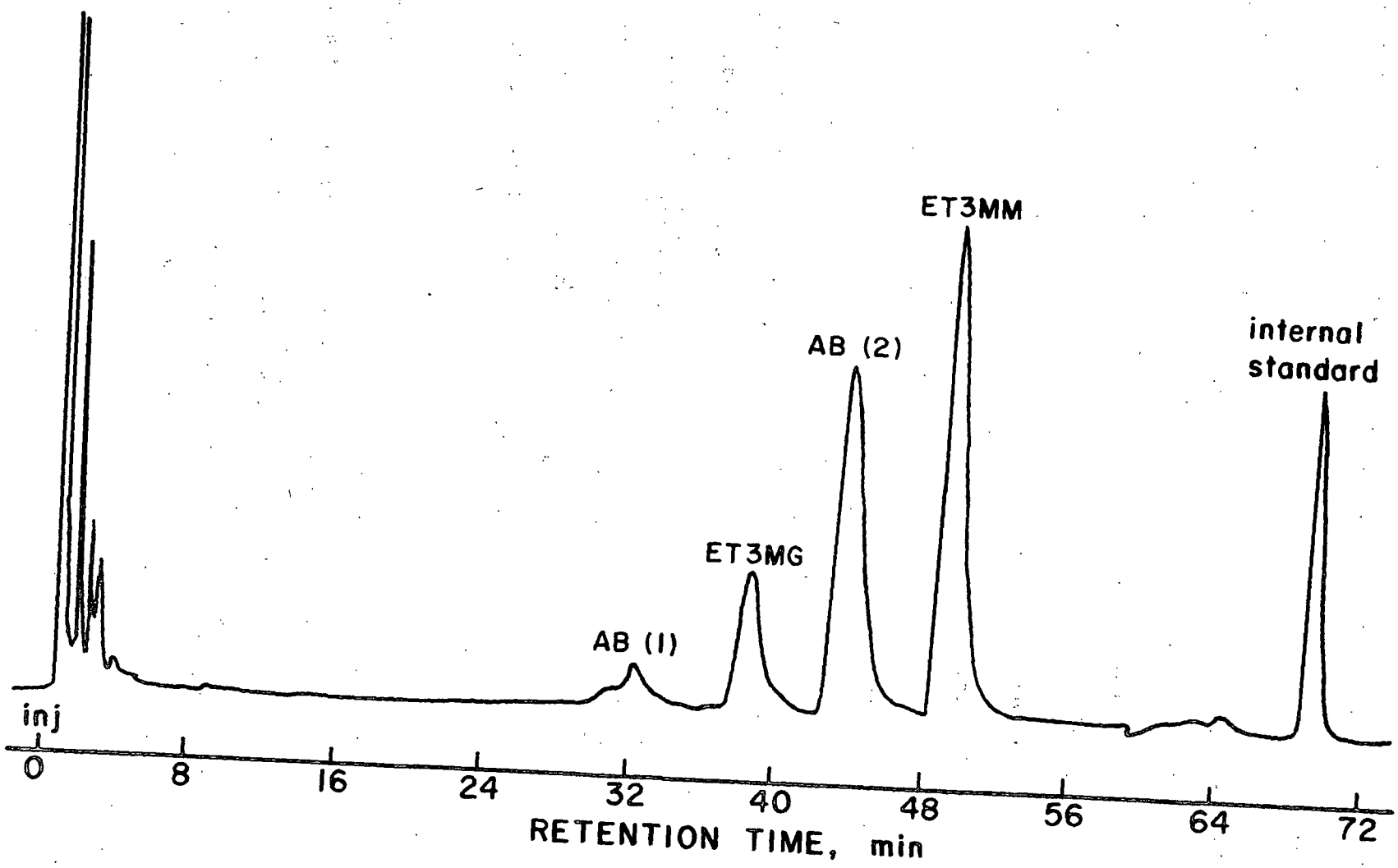


Figure 32. Sample Chromatogram of the Products of the Reduction of a Kinetic Sample During the Degradation of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose (0.10M) in 0.05N NaOH at 25°C. Peak AB (1) and AB (2) are the Results of α,β-KG Reduction and Peaks ET3MG and ET3MM are the Results of ET3M-KG Reduction

sample (0.1 mL) was diluted to 25 mL with triply-distilled water. The absorbance of this solution at 260 nm was measured against a distilled water reference within 1 minute of sampling the reaction.

The concentration of α,β-KG was obtained from a Beer-Lambert calibration curve (Fig. 33). The only absorption maximum between 220 nm and 400 nm was at 263 nm, indicating that there was no interference by other chromophores. Although hydrogen peroxide absorbs continuously in the UV region below 400 nm, its extinction coefficient at 260 nm is only 13 (61). Therefore, the absorbance from residual hydrogen peroxide was negligible.

Other Nonvolatile Products

Reaction samples (4 mL for ET3M-KG reactions and 1 mL for α,β-KG reactions) were neutralized with IR-120 (2 mL/mL reaction solution) that was thoroughly washed with acetone. The resin was filtered and washed successively with water (4 x 10 mL) and acetone (3 x 10 mL). The filtrates were concentrated *in vacuo* to a sirup. The last traces of water were removed by azeotropic evaporation of 1,2-dichloroethane. The yellow sirup was stored at *ca.* 2°C until derivatized. All concentrations were performed at bath temperatures of less than 40°C.

A portion (20-30 mg) of the product mixture was transferred to a 4 mL vial fitted with an air tight, Teflon-backed septum. The sample was dissolved in anhydrous chloroform (0.2 mL) and derivatized with the addition of Tri-Sil concentrate (0.2 mL) at 50°C for 12 hours. The solution was analyzed by GLC under conditions described in Appendix I.

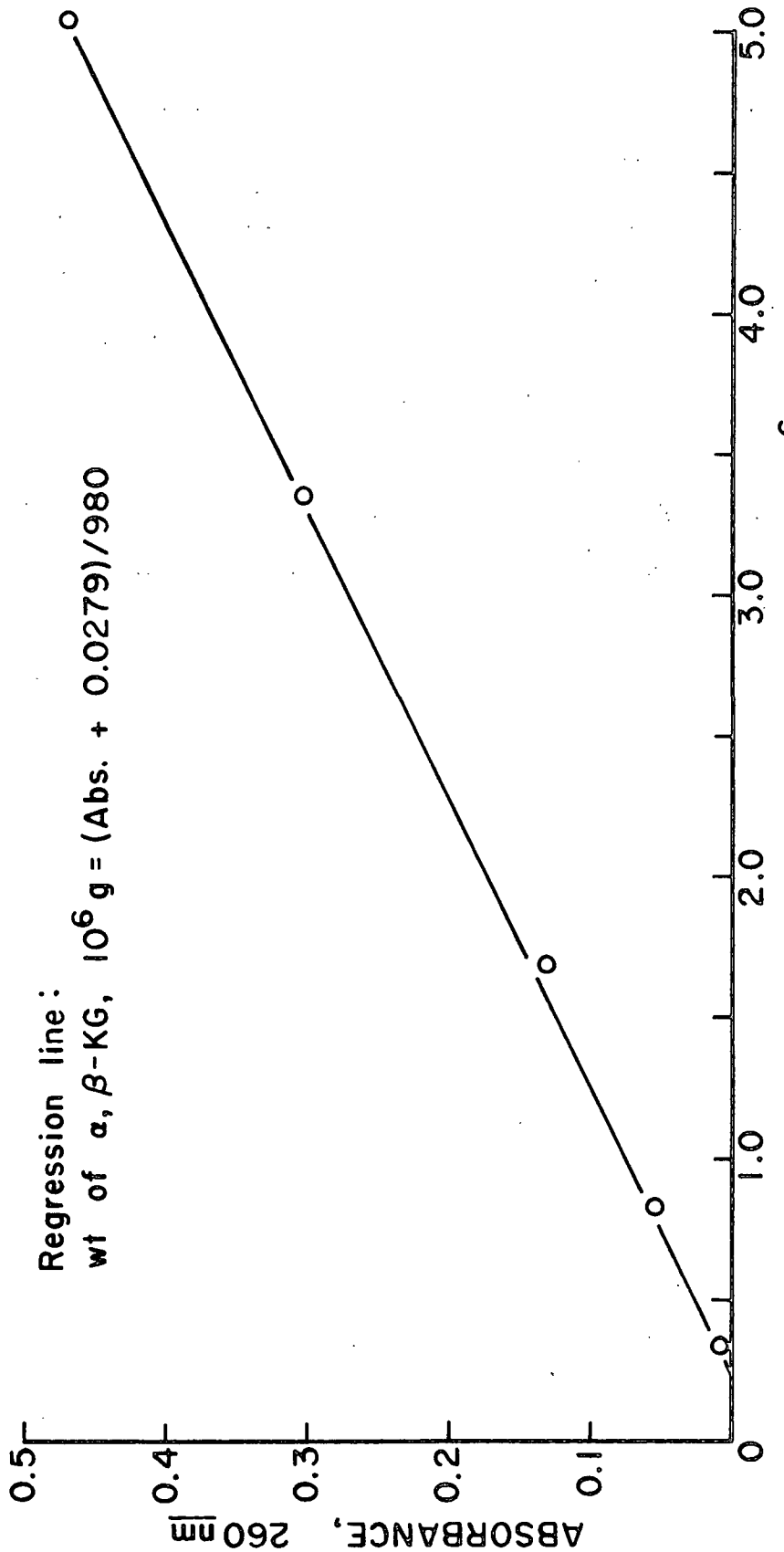


Figure 33. Beer-Lambert Calibration Curve for α,β-KG in Aqueous Solution

Preparative GLC Technique

The derivatized product mixture was separated by GLC (Appendix I) on a nickel column. A fraction of the column effluent stream was routed to a flame ionization detector; the remainder of the effluent stream was condensed in glass collection tubes that were packed with glass wool to prevent losses due to the formation of aerosols. The collection tubes were chilled in an ice bath to assist in condensing the sample. Each product was collected from several injections of the sample. Between collections the glass tubes were wrapped with Parafilm and stored in a desiccator over calcium chloride. The isolated product was washed from the glass tube with chloroform-d into NMR capillary tubes and analyzed by FT-^{1}H-NMR and/or FT-^{13}C-NMR immediately.

ACKNOWLEDGMENTS

The generous financial support of The Institute of Paper Chemistry and its member companies is gratefully acknowledged. Without that support, this work could not have been accomplished.

The author would also like to acknowledge the guidance provided by his thesis advisory committee: Drs. L. R. Schroeder, N. S. Thompson, and E. E. Dickey. The contribution of Dr. D. C. Johnson during the early portion of this work is also acknowledged. Special thanks are extended to Dr. L. R. Schroeder, the Thesis Chairman, for his assistance and encouragement.

To the rest of the faculty and staff at the Institute who have in many ways contributed to this work, the author gratefully extends his appreciation.

Thanks are also extended to three of the author's fellow students: David O. Hearne, Folke L. A. Arbin and Timothy J. Baker, for their assistance during the experimental portion of this work. The author would also like to thank John C. Gast for his help with the assignment of ^{13}C-NMR signals.

The author would like to thank all of his fellow students, their spouses and families, who have made his stay at the Institute very enjoyable.

Before I use up all of my space, I'd like to extend personal thanks to my families for their continued interest and encouragement. Extra special thanks go to my wife, Gretchen, for her assistance with the preparation of this manuscript, and, more importantly, for the never ending strength of spirit which she displayed throughout this ordeal which made my work much easier.

LITERATURE CITED

1. Weaver, J. D. The alkaline peroxide degradation of methyl β-D-glucopyranoside and methyl 4-O-methyl-β-D-glucopyranoside. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1975. Weaver, J. D., Schroeder, L. R., and Thompson, N. S., Carbohyd. Res. 48:C5-7(1976).

2. Millard, E. C. The degradation of selected 1,5-anhydroalditols by molecular oxygen in alkaline media. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1976. Millard, E. C., Schroeder, L. R., and Thompson, N. S., Carbohyd. Res. 56:259-76(1977).

3. Brown, N., Hartig, M. J., Roedel, M. J., Anderson, A. W., and Schweitzer, C. E., J. Am. Chem. Soc. 77:1756-9(1955). Brown, N., Anderson, M. J., and Schweitzer, C. E., J. Am. Chem. Soc. 77:1760-1(1955).

4. Milas, N. A., Harris, S. A., and Panagiotakos, P. C., J. Am. Chem. Soc. 61:2430-2(1939).

5. Milas, N. A., and Golubovic, A., J. Am. Chem. Soc. 81:3361-4, 5824-6, 6461-2 (1959).

6. Sinkey, J. D. The function of magnesium compounds in an oxygen-alkali-carbohydrate system. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1973. Sinkey, J. D., and Thompson, N. S., Papier Puu 56(5):473-86(1974).

7. McCloskey, J. T. The degradation of methyl β-D-glucopyranoside by oxygen in alkaline solution. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1971.

8. Gagedois, A., and Lille, D. B., Ger. pat. 191,766(June 29, 1905); CA 2: 1636(1908).

9. TAPPI Monograph No. 27. Bleaching of pulp. p. 180-98. New York, TAPPI, 1963.

10. Beeman, L. A., and Reichert, J. S. Peroxides in pulp bleaching processes. Wilmington, Delaware, Du Pont, 1951. 106 p.; and references cited therein.

11. Peroxide process for bleaching groundwood. Wilmington, Delaware, Du Pont, 1946. 29 p.; and references cited therein.

12. Ericsson, B., Lindgren, B. O., and Theander, O., Cellulose Chem. Technol. 8:363-85(1974).

13. Ericsson, B., and Malinen, R., Cellulose Chem. Technol. 8:327-38(1974).

14. Minor, J. L., and Sanyer, N., J. Polymer Sci., Part C, 36:73-84(1971).

15. Haskins, J. F., and Hogsed, M. J., J. Organic Chem. 15:1264-74(1950).

16. Isbell, H. S., J. Res. Natl. Bur. Stand. 32:45-59(1944).

17. Lewin, M., and Ettinger, A., Cellulose Chem. Technol. 3:9-20(1969).

18. Samuelson, O., and Thede, L., Tappi 52:99-104(1969).

19. Kolmodin, H., Carbohyd. Res. 34:227-32(1974).

20. Theander, O., Acta Chem. Scand. 12(10):1897-1905(1958).

21. Theander, O., Acta Chem. Scand. 12:1887-96(1958).

22. Hearne, D. O. The effect of the aglycone and hydroxyl orientation on alkali-oxygen degradations of methyl glycosides. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1978.

23. Theander, O., Tappi 48:105-10(1965).

24. Kenne, L., and Svensson, S., Acta Chem. Scand. 26(5):2144-6(1972).

25. Hawkins, E. G. S. Organic peroxides. Princeton, N.J., Van Nostrand Co., 1961. 434 p.

26. Isbell, H. S., Frush, H. L., and Martin, E. T., Carbohyd. Res. 26:287-95 (1973).

27. Isbell, H. S., and Frush, H. L., Carbohyd. Res. 28:295-301(1973).

28. Isbell, H. S., and Naves, R. G., Carbohyd. Res. 36:C1-4(1974).

29. Isbell, H. S., Frush, H. L., and Orhanovic, Z., Carbohyd. Res. 43:93-100 (1975).

30. Ericsson, B. Oxygen oxidation of cellulose in aqueous alkaline medium. Doctor's Dissertation. Stockholm, Sweden, Royal University of Technology, 1974.

31. Pappas, S. P., and Boa, L. Q., J. Am. Chem. Soc. 95(23):7906-7(1973).

32. House, H. O., and Ro, R. S., J. Am. Chem. Soc. 80:2428-33(1958).

33. Zimmerman, H. E., Singer, L., and Thyagarajan, B. S., J. Am. Chem. Soc. 81:108-16(1959).

34. Schroeder, L. R., Hultman, D. P., and Johnson, D. C., J. Chem. Soc., Perkin II, 72:1063-71(1972).

35. Beving, H. F. G., and Theander, O., Acta Chem. Scand. B, 29(6):641-6(1975).

36. Noller, C. R. Chemistry of organic compounds, 2nd Ed., p. 813. Philadelphia, Penn., W. B. Saunders Co., 1957.

37. Hurd, C. D. The pyrolysis of carbon compounds. p. 556-9. Am. Chem. Soc. Monograph Ser., New York, N.Y., The Chemical Catalog Co., Inc., 1929.

38. Dickens, F., Armand, G., Kon, R., and Thorpe, J. F., J. Chem. Soc. 121: 1496-1506(1922). Wislicenus, N., Chem. Ber. 27:792-5(1948).

39. Scott, A. I. Interpretation of the ultraviolet spectra of natural products. New York, N.Y., MacMillan, 1964. 443 p.

40. Silverstein, R. M., and Bassler, G. C. Spectrometric identification of organic compounds. 2nd Ed. p. 148-69. New York, N.Y., Wiley, 1967.

41. Satterfield, C. N., and Bonnell, A. H., Anal. Chem. 27:1174-5(1955).

42. Marklund, S., Acta Chem. Scand. 25:21-3(1971).

43. Pobiner, H., Anal. Chem. 33(10):1423-6(1961).

44. Bauer, N., and Lewin, S. Z. In Weisberger's Techniques in organic chemistry. Vol. I. Part 1. p. 136, New York, N.Y., Interscience, 1959.

45. Lund, H., and Bjerrum, J., Chem. Ber. 64:210-13(1931).

46. Reynolds, D. D., and Evans, W. L., J. Am. Chem. Soc. 60:2559-61(1938).

47. Ouellette, R. J., Can. J. Chem. 43(3):707-9(1965).

48. Reiner, D., and Poe, D. P., Anal. Chem. 49(6):889-91(1977).

49. Monzingo, R., Organic Syntheses 26:77-82(1946).

50. Swinehart, J. S. Organic chemistry: an experimental approach. p. 418. New York, N.Y., Meredith, 1969.

51. Bates, F. J. Polarimetry, saccharimetry and the sugars. p. 500. Washington, D.C., U.S. Government Printing Office, 1947.

52. Schroeder, L. R., and Green, J. W., J. Chem. Soc., Part C, 66:530-1(1966).

53. Ferguson, J., Toepffer, H., and Anderson, C. C., Chem. Ber. 61:1750-60 (1928).

54. Brimacombe, J. S., Jones, B. D., Stacey, M., and Willard, J. J., Carbohyd. Res. 2:167-9(1966).

55. Bell, D. J., and Lorber, J. J., J. Chem. Soc. 40:453-5(1940).

56. Ekborg, G., Lindberg, B., and Lönngren, J., Acta Chem. Scand. 26(8):3287-92 (1976).

57. Lemieux, R. U., and Cipera, J. D. T., Can. J. Chem. 34(7):906-10(1956).

58. Dykes, C. A. Acid-catalyzed reactions of 1,2-O-(1-exo-ethoxyethylidene)-3,4,6-tri-O-methyl-β-D-mannopyranose with ethanol. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1975.

59. Sims, E. W., J. Chromat. Sci. 14:65-7(1976).

60. Kolthoff, I. M., and Sandell, E. B. Textbook of quantitative inorganic chemistry. 3rd Ed. p. 574. New York, N.Y., MacMillan, 1956.

61. Schumb, W. C., Satterfield, C. N., and Wentworth, R. L. Hydrogen peroxide. p. 291. New York, N.Y., Reinhold, 1955.

62. FMC Corporation, The Operation of a Bench Scale Peracetic Acid Generator, New York, 1963.

APPENDIX I

GLC ANALYSIS

Quantitative GLC was accomplished by correcting for differences in detector response between equimolar amounts of reaction component and the appropriate internal standard. Molar response correction factors were calculated according to Equation (1),

$$f_x = (A_s/A_x)(M_x/M_s), \qquad (1)$$

where f_x = molar response factor for Compound X as measured against the selected internal standard, s

A_s = peak area for internal standard

A_x = peak area for Compound X

M_x = moles of Compound X in the solution being analyzed

M_s = moles of internal standard in the solution being analyzed

Each response factor was experimentally determined from no fewer than four solutions consisting of varying molar ratios of Compound X and the appropriate internal standard. These solutions were treated in the same manner as samples taken from reaction solutions (see Experimental), and analyzed by GLC with no fewer than triplicate injections. The response factor was the average of the values.

The determination of the response factor for ET3M-KG was complicated by the fact that ET3M-KG was analyzed as its sodium borohydride reduction products. In this case, individual response factors for ET3MG and ET3MM were independently determined without being treated through the sample work-up procedure. Subsequently, solutions of ET3M-KG and internal standard were reduced with sodium borohydride and treated through the sample work-up procedure. These solutions

were then analyzed by GLC and the response factor of ET3M-KG was calculated from Equation (2):

$$f_{kg} = M_{kg}/(M_s\ B), \qquad (2)$$

where $B = (f_{ET3MG}\ A_{ET3MG}) + (f_{ET3MM}\ A_{ET3MM})$,

and f_{kg} = molar response factor of ET3M-KG

M_{kg} = moles of ET3M-KG in the solution being analyzed

f_{ET3MG} = molar response factor of ET3MG

A_{ET3MG} = peak area ratio of ET3MG to internal standard

f_{ET3MM} = molar response factor of ET3MM

A_{ET3MM} = peak area ratio of ET3MM to internal standard

Table V lists the conditions used in GLC analyses in this work, and Table VI shows GLC retention times and response factors (where possible) for the compounds involved with this work.

The response factor was used to calculate the concentration of Compound X in the reaction solution by using Equation (3).

$$[X] = f_x\ M_s\ (A_x/A_s)\ 1000/S, \qquad (3)$$

where [X] = concentration of Compound X, moles liter^{-1}

S = reaction sample volume, mL

In special Case 1, where X = ET3M-KG, Equation (4) was used to obtain the concentration of ET3M-KG in the reaction solution.

$$[kg] = f_{kg}\ M_s\ B\ 1000/S \qquad (4)$$

In special Case 2, where X = α,β-KG, Equation (5) was used to determine the concentration of α,β-KG during the alkaline degradation of ET3M-KG.

TABLE V

GAS-LIQUID CHROMATOGRAPHIC CONDITIONS

Conditions	A	B	C	D	E	F	G
Analysis	ET3M-KG	ET3MG	Alcohols	Products	Products	Products Prep-GLC	Products Prep-GLC
Column type	OV-17[a]	OV-17[a]	Carbowax[b]	SP-2250[c]	OV-17[a]	OV-17[d]	OV-17[e]
Derivative	none	none	none	TMS	TMS	TMS	TMS
Column temperature programming, °C	130→182 @ 1°/min 182→210 @ 4°/min	175→225 @ 2°/min	65→95 @ 1°/min	170	70→200 @ 2°/min	70→200 @ 2°/min	170
Injector temperature, °C	250	250	115	200	200	260	200
Detector temperature, °C	300	300	315	300	300	260	200
N_2 flow rate, (mL min^{-1})	30	30	30	20	20	84	84

[a] 5% OV-17 on 80/90 mesh Anachrome ABS (10 ft x 0.125 inch o.d. stainless steel column rigged for on-column injection).

[b] 5% Carbowax 20M on 80/100 mesh Chromosorb 101 (4 ft x 0.125 inch o.d. stainless steel column rigged for off-column injection).

[c] 3% SP-2250 on 100/120 mesh Suplecoport (14 ft x 0.125 inch i.d. all glass column rigged for on-column injection).

[d] 5% OV-17 on 80/90 mesh Anachrome ABS (10 ft x 0.25 inch o.d. stainless steel column rigged for off-column injection).

[e] 3% OV-17 on 80/100 mesh Suplecoport (20 ft x 0.25 inch o.d. nickel column rigged for off-column injection).

TABLE VI

RETENTION TIMES (T_r) AND RESPONSE FACTORS (f_x)

Conditions	Compound	T_r, min	f_x
A	AB(1)	28.0-34.0	
	ET3MG	38.5	1.143 ± 0.002[a]
	AB(2)	41.0-47.0	
	ET3MM	48.5	1.198 ± 0.004[a]
	ET3M-KG		1.060 ± 0.010[a]
	CT3MG	73.0	1.000[a]
B	ET3MG	7.5	1.398 ± 0.063[a]
	CT3MG	23.7	1.000[a]
C	Methanol	3.6	2.970 ± 0.019[b]
	Ethanol	7.7	2.113 ± 0.056[b]
	n-Propanol	19.1	1.000[b]
D	Unknown A	14.5	
	Unknown B	17.3	
	Unknown C	18.4	
	Unknown D	20.1	
	Unknown E	22.6	
	α,β-KG	25.0	3.73 ± 0.01[c]
	Ethyl 4,6-di-O-methyl-β-D-glucopyranoside	32.9	1.000[c]
	Unknown H	20.8	

[a]Calculated relative to cyclohexyl 3,4,6-tri-O-methyl-β-D-glucopyranoside at the specified conditions.

[b]Calculated relative to n-propanol as internal standard at the specified conditions.

[c]Calculated relative to ethyl 4,6-di-O-methyl-β-D-glucopyranoside as internal standard at the specified conditions.

$$(\alpha,\beta) = M_s \ (AB(1)/A_s + AB(2)/A_s) \ 1000/S, \qquad (5)$$

where AB(1) = area under the first series of peaks resulting from the reduction of α,β-KG

AB(2) = area under the second series of peaks resulting from the reduction of α,β-KG

APPENDIX II

CONSTRUCTION OF PEROXIDE CALIBRATION CURVE

A straight line calibration curve (Fig. 34) was constructed by analyzing various hydrogen peroxide solutions by a standard iodometric titration procedure (60) and by the titanium sulfate colorimetric method described earlier in this report (Experimental section).

The following standard iodometric procedure was used for this work. It was adapted from the one described by Kolthoff and Sandell (60).

An aliquot of test solution was added to 4N sulfuric acid (20 mL) in an Erlenmeyer flask (250 mL). The dissolved oxygen in the solution was displaced by CO_2 generated with the addition of sodium carbonate (2 x ca. 0.25 g) and gently swirling. Three drops of 3% ammonium molybdate catalyst were added prior to the addition of 10% potassium iodide (10 mL, w/v). The solution was then immediately titrated with standardized sodium thiosulfate solution to a starch end point. The concentration of hydrogen peroxide was calculated from Equation (6).

$$[\text{Peroxide}] = V_t \, C_t / 2 \, V_s, \qquad (6)$$

where V_t = volume of sodium thiosulfate used, mL

C_t = concentration of sodium thiosulfate solution, moles/mL

V_s = volume of test sample used, mL

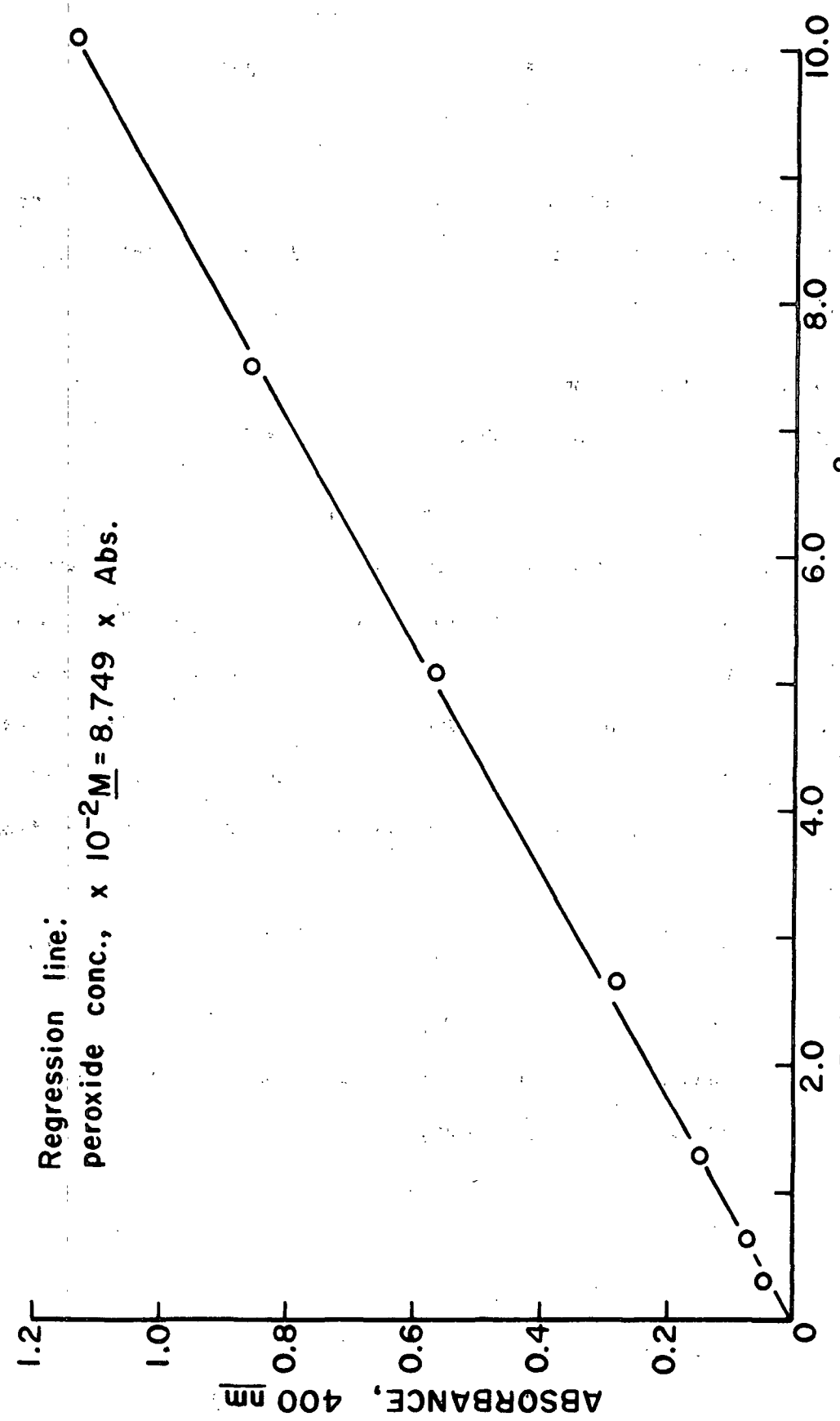


Figure 34. Absorbance at 400 nm vs. Peroxide Concentration as Determined by a Standard Titration Method

APPENDIX III

EXPERIMENTAL DATA

TABLE VII

DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-*arabino*-HEXOPYRANOSIDULOSE (0.10M) IN 0.05N NaOH AT 25°C

Time, min	ET_3M-KG, x 10^{-2}M	α,β-KG, x 10^{-2}M	MeOH, x 10^{-2}M	EtOH, x 10^{-2}M
0.00	10.04	0.00	0.00	0.00
1.67	1.32	5.21	9.18	0.37
2.75	0.74	4.18	10.70	0.71
4.00	0.38	5.39	10.80	0.90
8.00	0.00	6.68	11.90	1.92
12.00	0.00	6.28	11.70	2.45
16.00	0.00	6.48	11.70	2.99
20.00	0.00	5.29	12.30	3.86
40.00	0.00	4.81	12.30	5.05
60.00	0.00	4.35	12.60	5.80

TABLE VIII

DEGRADATION OF ETHYL 4-DEOXY-3,6-DI-O-METHYL-β-D-*glycero*-HEX-3-ENOPYRANOSIDULOSE (0.10M) IN 0.05N NaOH AT 25°C

Time, min	α,β-KG, x 10^{-2}M	MeOH, x 10^{-2}M	EtOH, x 10^{-2}M
0.00	10.19	0.00	0.00
1.50	N.D.[a]	4.70	1.01
2.00	9.12	3.86	1.12
4.00	8.56	3.42	1.80
7.00	N.D.[a]	4.02	2.40
8.00	7.64	N.D.[a]	N.D.[a]
12.00	7.36	3.40	3.19
18.00	6.30	4.81	4.48
25.00	5.69	3.89	4.72
40.00	5.65	3.70	5.06
60.00	5.42	3.55	5.67

[a] Not determined.

TABLE IX

DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-*arabino*-HEXOPYRANOSIDULOSE (0.10M) IN 0.05N NaOH AND 0.10M H_2O_2 AT 25°C

Time, min	ET3M-KG, x 10^{-2}M	α,β-KG, x 10^{-2}M	MeOH x 10^{-2}M	EtOH x 10^{-2}M
0.00	10.00	0.00	0.00	0.00
1.00	N.D.[a]	4.39	N.D.[a]	N.D.[a]
1.25	4.42	N.D.[a]	N.D.[a]	N.D.[a]
2.17	4.45	N.D.[a]	N.D.[a]	N.D.[a]
3.00	N.D.[a]	4.95	N.D.[a]	N.D.[a]
4.00	3.94	N.D.[a]	7.24	1.00
5.00	N.D.[a]	4.73	N.D.[a]	N.D.[a]
7.00	3.41	5.08	7.73	0.94
10.00	N.D.[a]	4.99	N.D.[a]	N.D.[a]
12.00	3.75	N.D.[a]	8.70	1.18
15.00	N.D.[a]	5.01	N.D.[a]	N.D.[a]
18.00	3.24	N.D.[a]	8.15	1.31
22.00	N.D.[a]	5.07	N.D.[a]	N.D.[a]
26.00	3.03	N.D.[a]	9.16	1.43
30.00	N.D.[a]	5.33	N.D.[a]	N.D.[a]
40.00	2.99	5.09	8.70	1.39
60.00	2.94	5.40	8.08	1.88

[a]Not determined.

TABLE X

DEGRADATION OF ETHYL 4-DEOXY-3,6-DI-O-METHYL-β-D-*glycero*-HEX-3-ENOPYRANOSIDULOSE (0.10M) IN 0.05N NaOH AND 0.10M H_2O_2 AT 25°C

Time, min	α,β-KG, x 10^{-2}M	MeOH, x 10^{-2}M	EtOH, x 10^{-2}M
0.00	10.01	0.00	0.00
1.50	9.23	2.47	0.99
3.00	8.93	2.94	1.26
6.00	8.89	2.74	1.54
10.00	7.50	3.25	1.49
15.00	8.39	3.19	1.97
22.00	8.66	2.96	1.77
30.00	8.33	3.62	2.84
45.00	8.58	3.31	1.91
60.00	8.15	3.21	1.86

TABLE XI

DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-*arabino*-HEXOPYRANOSIDULOSE (0.10M) AT pH 9.5 IN SODIUM BICARBONATE BUFFER

Time, min	ET3M-KG, x 10^{-2}M
0.00	10.05
15.00	10.90
30.00	9.60
45.00	10.20
60.00	9.70

TABLE XII

DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-*arabino*-HEXOPYRANOSIDULOSE (0.058M) AT pH 8.0 IN SODIUM PHOSPHATE BUFFER

Time, min	ET3M-KG, x 10^{-2}M
0.00	5.76
37.00	6.93
75.00	6.28
135.00	6.52
1440.00	5.77

TABLE XIII

STABILITY OF HYDROGEN PEROXIDE IN ALKALINE SOLUTION (0.25N NaOH)

Time, min	H_2O_2 x 10^{-2}M
0.00	10.56
2.00	10.32
4.00	10.41
8.00	10.67
16.00	10.41
32.00	10.59
60.00	10.32
180.00	10.50

TABLE XIV

DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-GLUCOPYRANOSIDE (0.10M) NaOH AND 0.2M H_2O_2 AT 60°C

Time, min	ET3MG, x 10^{-2}M	MeOH, x 10^{-2}M	EtOH, x 10^{-2}M
0.00	11.70	0.000	0.000
5.00	11.40	0.143	0.122
15.50	11.20	0.375	0.187
36.00	10.80	0.308	0.202
60.00	10.80	0.378	0.292
91.00	10.20	0.288	0.268
120.00	10.60	0.317	0.169
150.00	10.20	0.187	0.168
180.00	11.50	0.179	0.151
240.00	12.90	N.D.[a]	N.D.[a]
300.00	10.70	0.270	0.208
360.00	10.70	N.D.[a]	N.D.[a]
423.00	10.10	0.286	0.229
476.00	10.50	N.D.[a]	N.D.[a]
592.00	12.00	0.288	0.237

[a]Not determined.

TABLE XV

PEROXIDE FORMATION DURING DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-GLUCOPYRANOSIDE (0.10M) IN 1.25N NaOH AND 0.20M H_2O_2 AT 60°C

Time, min	H_2O_2, x 10^{-2}M	Organic Peroxides, x 10^{-3}M
0.00	20.03	0.00
5.00	18.90	9.60
15.00	17.60	13.60
36.00	14.40	8.80
60.00	10.90	6.80
91.00	7.20	6.50
120.00	4.80	5.90
150.00	3.10	1.80
180.00	2.00	0.50
240.00	0.60	0.50
300.00	0.02	0.90
476.00	0.00	0.90

TABLE XVI

PEROXIDE FORMATION DURING THE DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-arabino-HEXOPYRANOSIDULOSE (0.10M) IN 0.05N NaOH AND 0.10M H_2O_2 AT 25°C

Time, min	H_2O_2, x 10^{-2}M	Organic Peroxides, x 10^{-3}M
0.00	10.60	0.00
2.75	4.79	3.67
5.00	4.30	2.71
8.00	3.88	2.19
13.00	3.67	2.36
17.00	3.66	0.52
25.00	3.50	0.35
45.00	3.44	1.31
	Duplicate Reaction	
0.00	10.30	0.00
2.25	5.92	0.00
5.50	4.43	0.00
8.00	3.89	0.00
12.00	3.45	0.02
16.00	3.26	0.04
25.00	3.17	0.07
45.00	3.05	0.08
	Triplicate Reaction	
0.00	9.90	0.00
1.00	7.86	3.15
3.00	5.07	2.62
6.00	4.19	2.10
10.00	3.88	2.01
15.00	3.88	0.61
21.00	3.83	2.71
28.00	3.71	1.75
36.00	3.71	2.01
45.00	3.74	2.19

TABLE XVII

PEROXIDE FORMATION DURING THE DEGRADATION OF ETHYL 4-DEOXY-3,6-DI-O-METHYL-β-D-glycero-HEX-3-ENOPYRANOSIDULOSE (0.10M) IN 0.05N NaOH AND 0.10M H_2O_2 AT 25°C

Time, min	H_2O_2, x 10^{-2}M	Organic Peroxides, x 10^{-3}M
0.00	10.21	0.00
2.50	5.10	1.60
4.00	4.58	1.80
6.00	4.45	1.80
9.00	4.34	1.50
12.00	4.25	1.10
15.00	4.20	0.90
30.00	4.12	0.80
	Duplicate Reaction	
0.00	10.36	0.00
2.00	5.41	1.14
5.00	4.86	1.05
7.00	4.80	0.96
11.00	4.58	1.05
16.00	4.51	0.96
24.00	4.44	0.96
31.00	4.50	0.35
46.00	4.61	0.09
61.00	4.52	0.26

TABLE XVIII

PEROXIDE FORMATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-arabino-HEXOPYRANOSIDULOSE (0.10M) IN 0.10M H_2O_2 AT 25°C

Time, min	H_2O_2, x 10^{-2}M	Organic Peroxides, x 10^{-3}M
0.00	10.00	0.00
1.75	10.05	0.35
3.00	10.08	0.61
5.00	10.04	0.57
8.00	10.06	0.13
12.50	10.14	1.71
18.00	10.17	0.70
25.00	10.04	1.31
36.00	9.92	1.18
45.00	9.97	1.18
60.00	10.00	1.62

PEROXIDE FORMATION OF ETHYL 4-DEOXY-3,6-DI-O-METHYL-β-D-glycero-HEX-3-ENOPYRANOSIDULOSE (0.10M) IN 0.10M H_2O_2 AT 25°C

Time, min	H_2O_2, x 10^{-2}M	Organic Peroxides, x 10^{-3}M
0.00	10.29	0.00
3.00	10.29	0.00
8.00	10.36	0.17
15.00	10.22	0.00
30.00	10.39	1.40
60.00	10.06	0.00

TABLE XX

DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-arabino-HEXOPYRANOSIDULOSE (0.10M) IN 0.05N NaOH AND 0.10M H_2O_2 AT 25°C

Time, min	Et_3M-kg, x 10^{-2}M
0.00	10.00
2.00	3.41
4.00	2.57
8.00	1.99
16.00	2.03
24.00	1.76
40.00	1.76
60.00	1.88
125.00	1.92
240.00	1.84
480.00	2.06
720.00	1.76
1080.00	1.95
1440.00	1.87
1860.00	1.88
2400.00	1.99
3000.00	1.74
3720.00	1.71
4740.00	1.72
6000.00	1.79
7260.00	1.87
8640.00	2.14
10080.00	2.01

TABLE XXI

DEGRADATION OF ETHYL 3,4,6-TRI-O-METHYL-β-D-*arabino*-HEXOPYRANOSIDULOSE (0.10M) IN 0.25N NaOH AND 0.10M H_2O_2 AT 25°C

Time, min	ET3M-KG, x 10^{-2}M	α,β-KG, x 10^{-2}M	H_2O_2, x 10^{-2}M	Organic Peroxides, x 10^{-2}M
1.16	3.12	2.37	N.D.[a]	N.D.[a]
2.08	1.23	2.48	N.D.[a]	N.D.[a]
2.50	N.D.[a]	N.D.[a]	3.75	0.07
4.00	0.229	1.93	N.D.[a]	N.D.[a]
5.00	N.D.[a]	N.D.[a]	0.79	0.17
7.00	--	1.73	N.D.[a]	N.D.[a]
7.75	N.D.[a]	N.D.[a]	--	0.10
10.25	N.D.[a]	N.D.[a]	--	0.15
12.00	--	1.38	N.D.[a]	N.D.[a]
15.25	N.D.[a]	N.D.[a]	--	0.28
17.00	--	0.95	N.D.[a]	N.D.[a]
25.00	N.D.[a]	N.D.[a]	--	0.25
26.00	--	0.552	N.D.[a]	N.D.[a]
40.00	--	0.173	N.D.[a]	N.D.[a]
45.00	N.D.[a]	N.D.[a]	0.02	0.24
60.00	--	0.0689	N.D.[a]	N.D.[a]

[a]Not determined.

APPENDIX IV

CALCULATION OF AVAILABLE HYDROXIDE IN ALKALINE PEROXIDE DEGRADATION OF ET3M-KG

1. $H_2O_2 \rightleftharpoons HOO^- + H^+$ $\quad pK_a = 11.75$

2. $H_2O \rightleftharpoons HO^- + H^+$ $\quad K_w = K_{eq}[H_2O] = [H^+][HO^-]$

3. $H_2O_2 + HO^- \rightleftharpoons H_2O + HOO^-$

$$K_3 = [H_2O][HOO^-]/[H_2O_2][HO^-] \qquad [HO^-] = K_w/[H^+]$$

$$K_1 = [HOO^-][H^+]/[H_2O_2] = 1.788 \times 10^{-12}$$

$$K_3 = [HOO^-][H^+]/[H_2O_2]\ [H_2O]/K_w = K_1\ [H_2O]/K_w$$

$$K_3 = 1.788 \times 10^{-12}\ 55.56/1 \times 10^{-14} = 9.879 \times 10^3$$

Initially: $[H_2O_2] = 0.1\underline{M}$; $[OH^-] = 0.05\underline{N}$

At equilibrium: $H_2O_2 = 0.1 - X$; $HO^- = 0.05 - X$; $HOO^- = X$;

$$[H_2O] = 55.56 + X$$

$$K_3 = 9.879 \times 10^3 = (55.56 + X)\ X/(0.1-X)(0.05-X) = 55.56X + X^2/0.005-0.15X+X^2$$

$$9879\ X^2 - 1482\ X + 49.4 = 55.56\ X + X^2$$

$$9878\ X^2 - 1538\ X + 49.4 + 0$$

$$X = 1538 \pm 1538^2 - 4\ (9878)(49.4)/2(9878)$$

$$X = 0.045$$

$$[H_2O_2] = 0.055\underline{M}$$

$$[HOO^-] = 0.045\underline{M}$$

$$[HO^-] = 0.005\underline{M}$$

$$[H^+] = 2.0 \times 10^{-12}\underline{M}$$

$$pH = 11.7$$

APPENDIX V

SELECTED SPECTRA

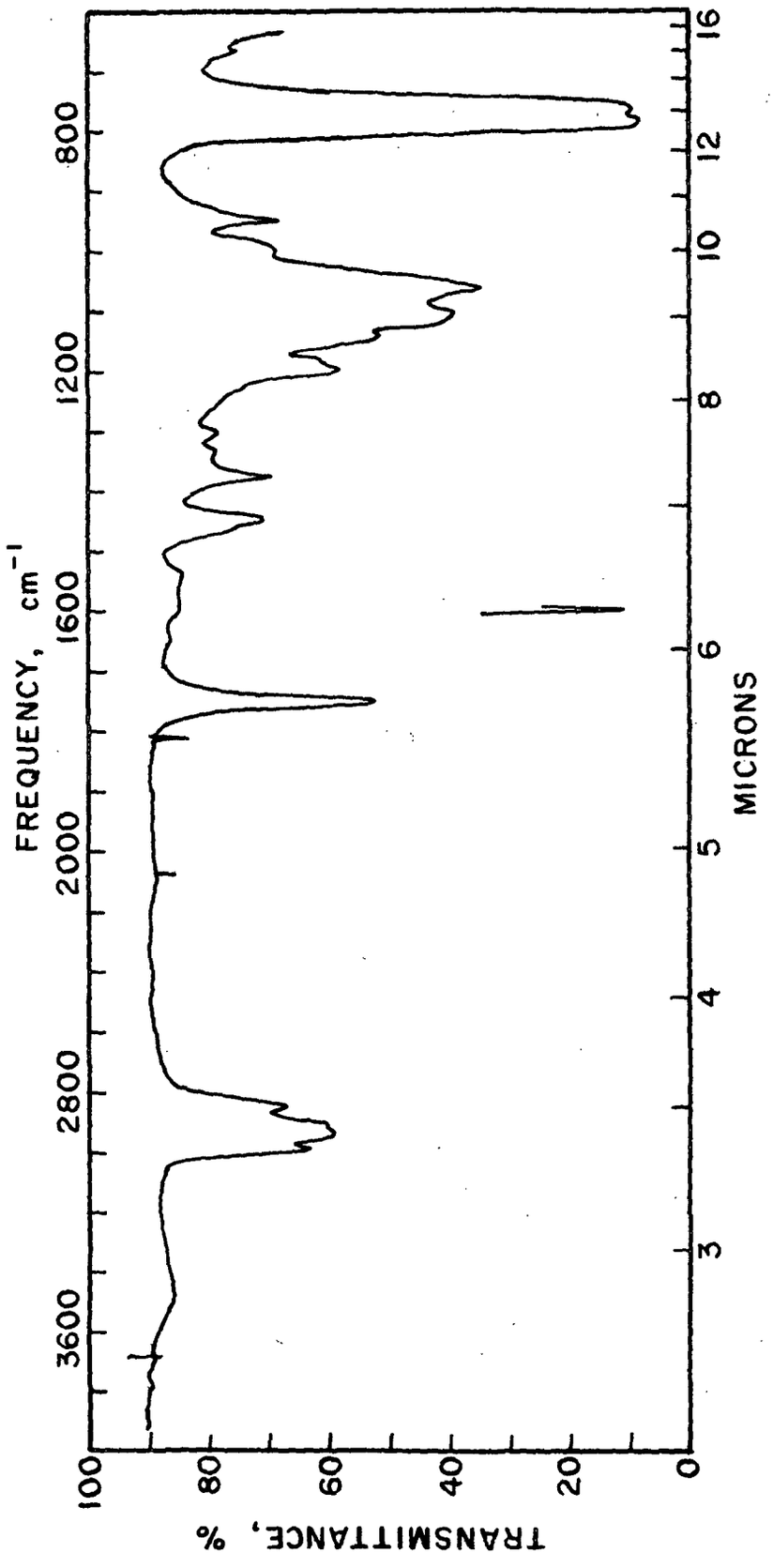
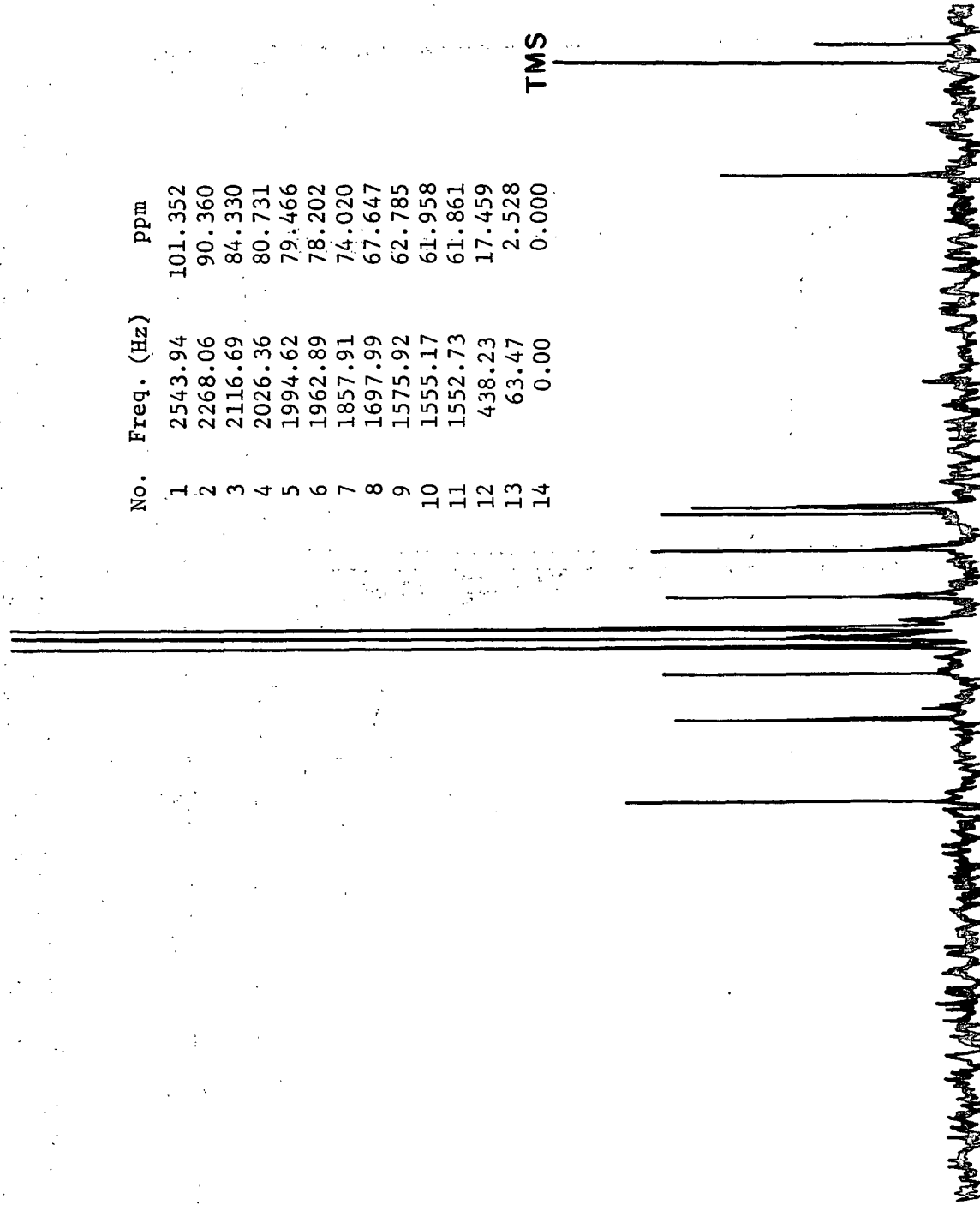


Figure 35. Infrared Spectrum of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose in Carbon Tetrachloride Solution



No.	Freq. (Hz)	ppm
1	2543.94	101.352
2	2268.06	90.360
3	2116.69	84.330
4	2026.36	80.731
5	1994.62	79.466
6	1962.89	78.202
7	1857.91	74.020
8	1697.99	67.647
9	1575.92	62.785
10	1555.17	61.958
11	1552.73	61.861
12	438.23	17.459
13	63.47	2.528
14	0.00	0.000

Figure 36. ^{13}C-NMR Spectrum of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose in Chloroform-d using Tetramethylsilane as Internal Reference. (The C-2 Signal has Folded Back Into the Spectrum and Appears to the Right of the TMS Signal.)

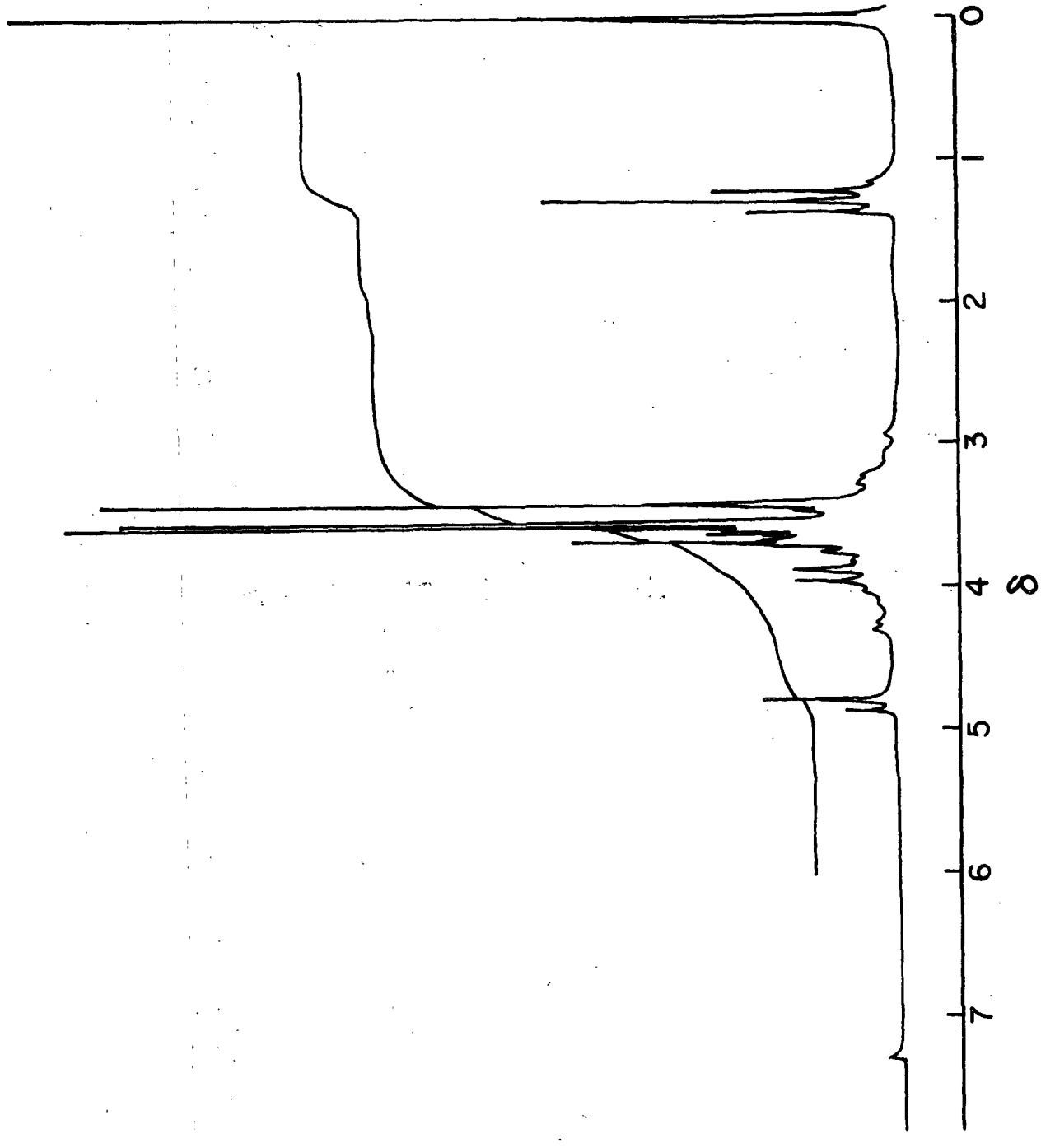


Figure 37. ^{1}H-NMR Spectrum of Ethyl 3,4,6-Tri-O-methyl-β-D-arabino-hexopyranosidulose in Chloroform-d Using Tetramethylsilane as an Internal Reference

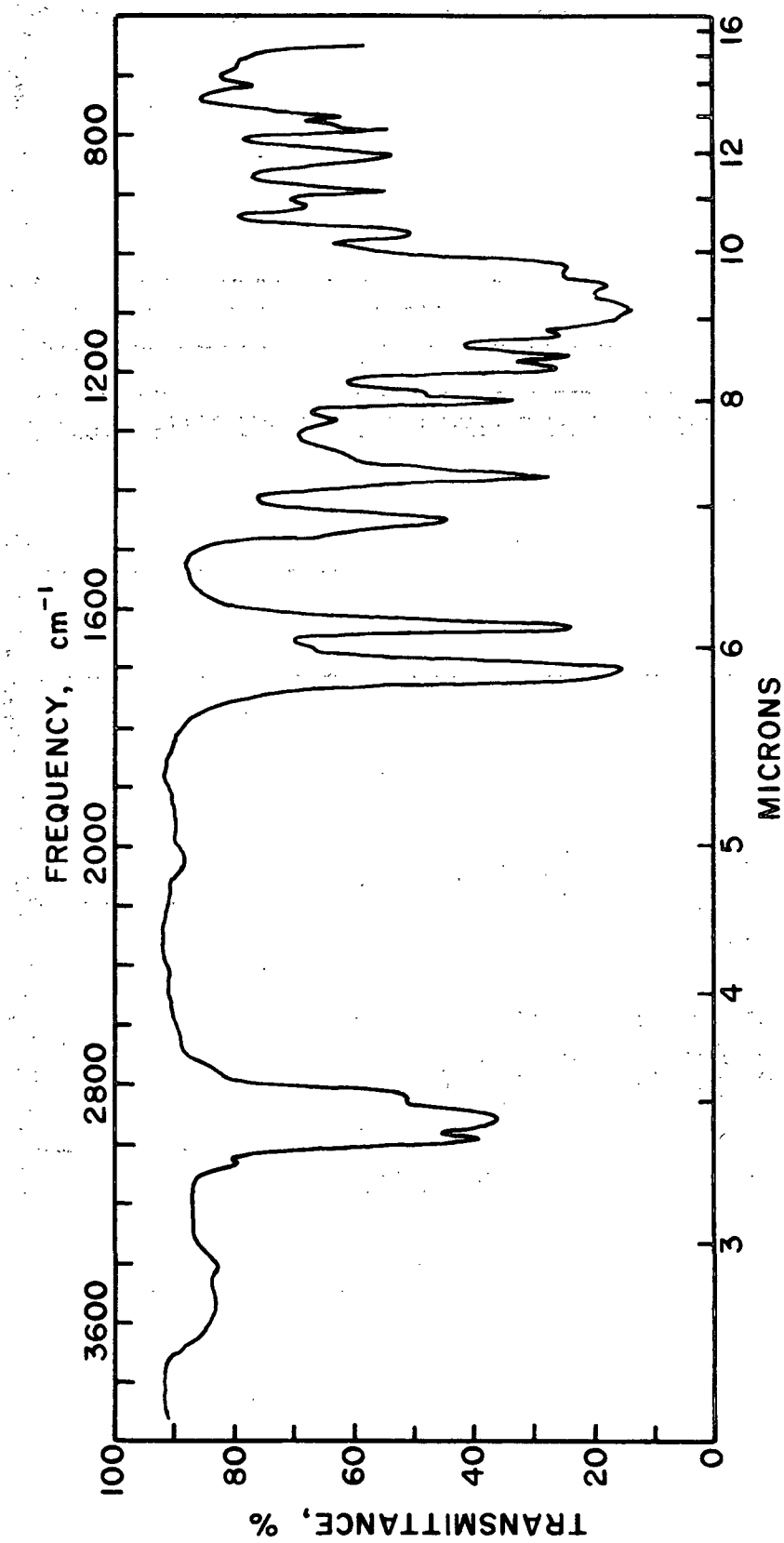
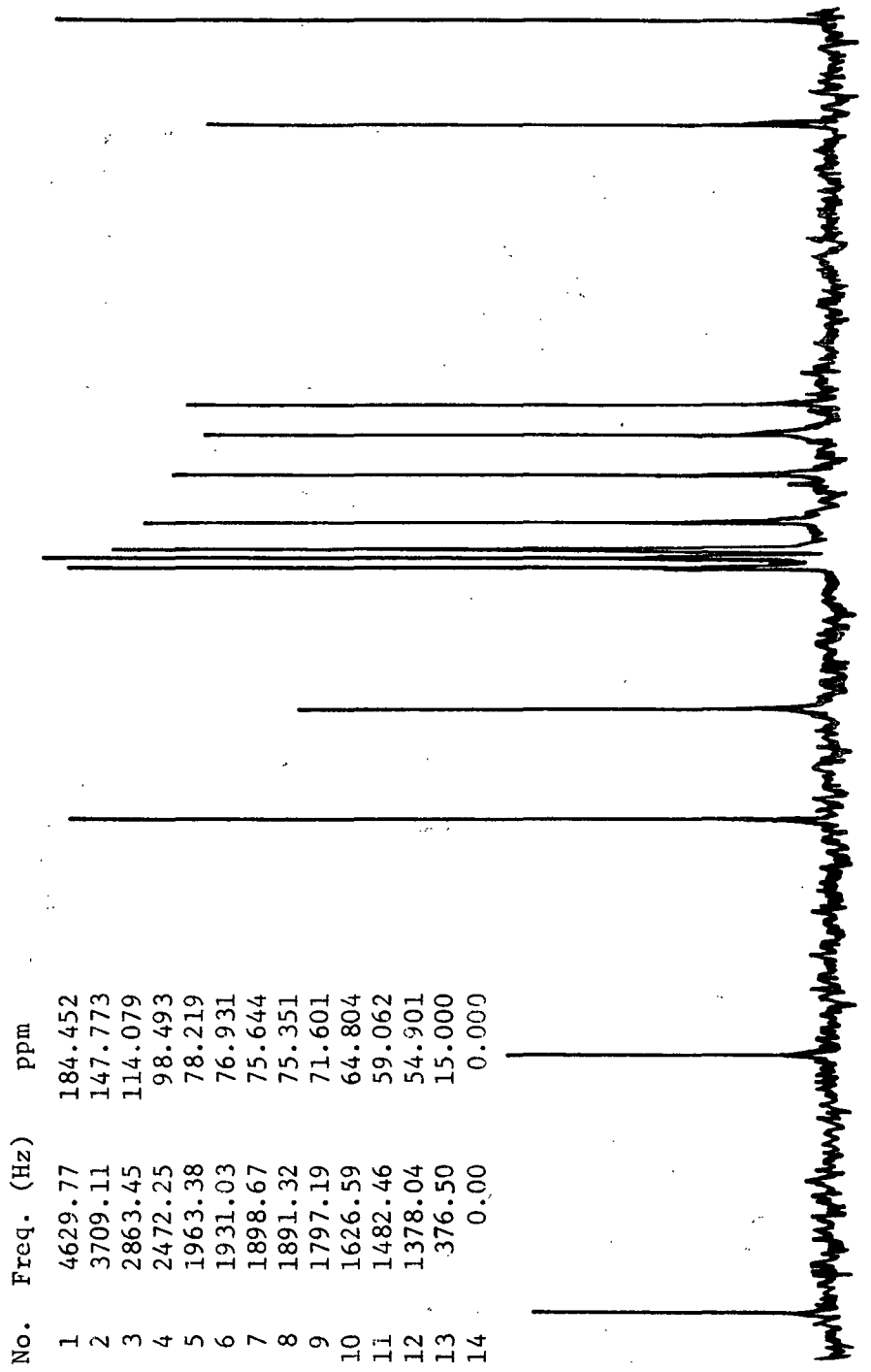


Figure 37. Infrared Spectrum of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose



No.	Freq. (Hz)	ppm
1	4629.77	184.452
2	3709.11	147.773
3	2863.45	114.079
4	2472.25	98.493
5	1963.38	78.219
6	1931.03	76.931
7	1898.67	75.644
8	1891.32	75.351
9	1797.19	71.601
10	1626.59	64.804
11	1482.46	59.062
12	1378.04	54.901
13	376.50	15.000
14	0.00	0.000

Figure 39. ^{13}C-NMR of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose in Chloroform-d Using Tetramethylsilane as Internal Reference

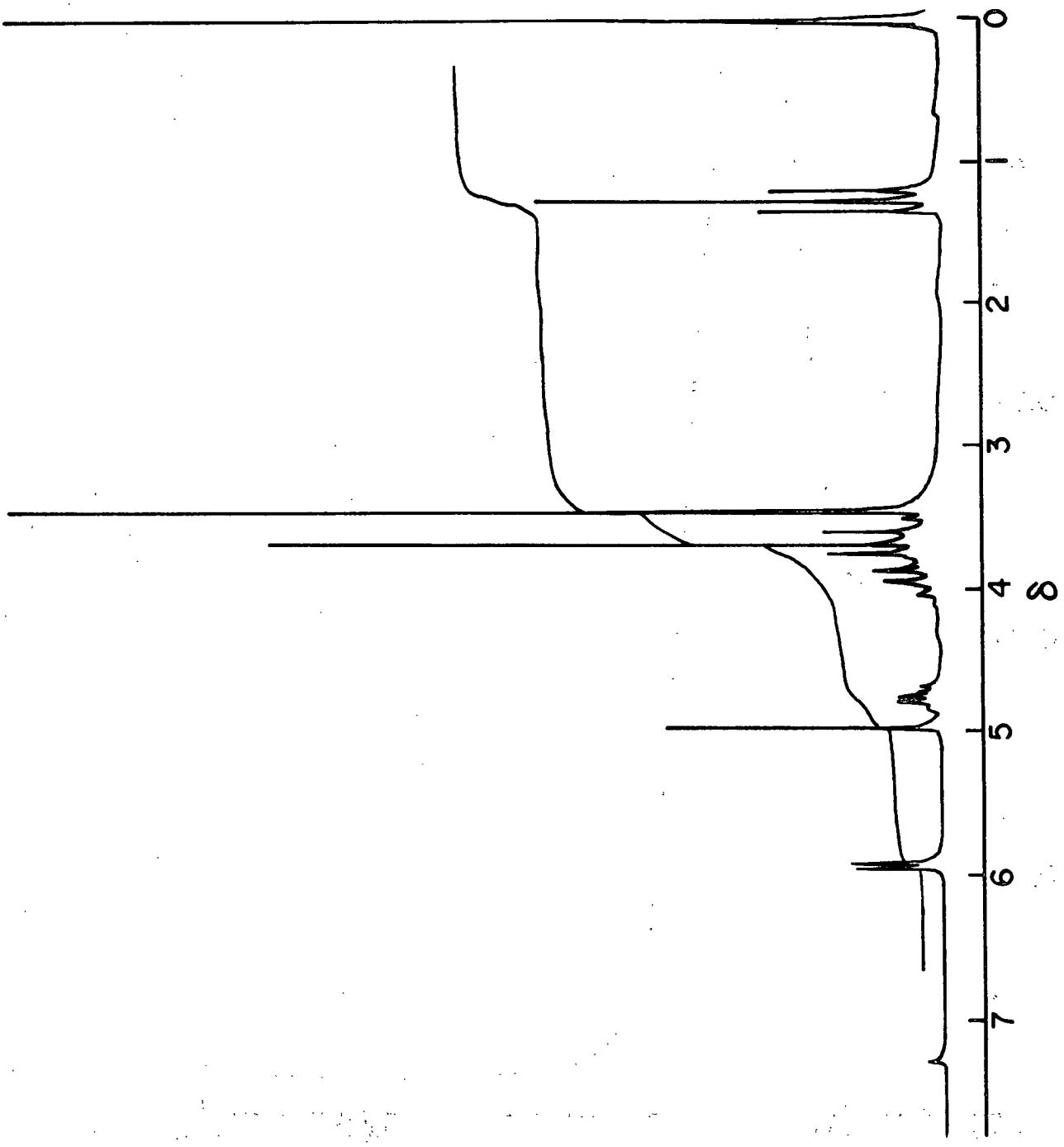


Figure 40. ^{1}H-NMR of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose in Chloroform-d Using Tetramethylsilane as Internal Reference

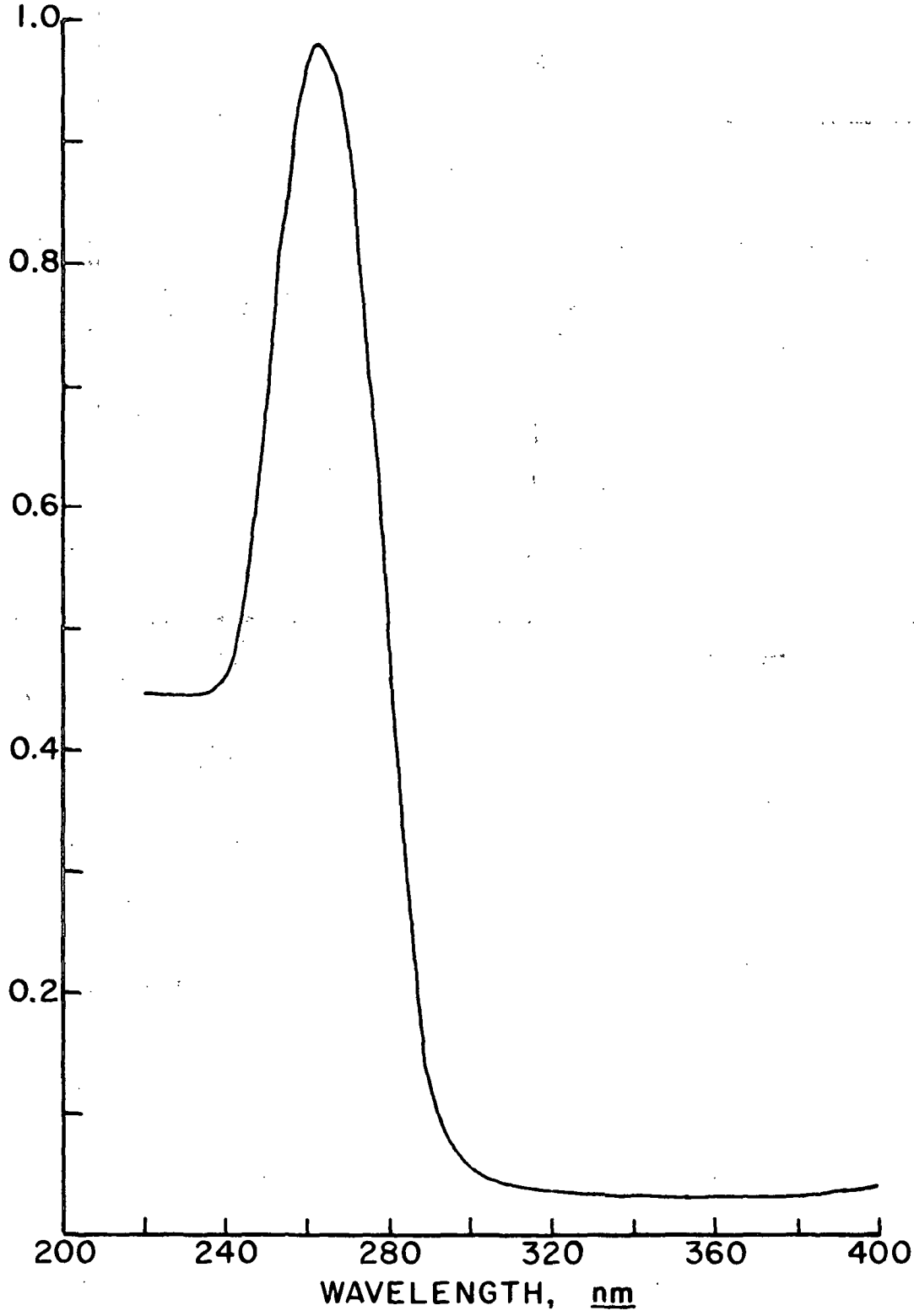


Figure 41. Ultraviolet Spectrum of Ethyl 4-Deoxy-3,6-di-O-methyl-β-D-glycero-hex-3-enopyranosidulose in Water

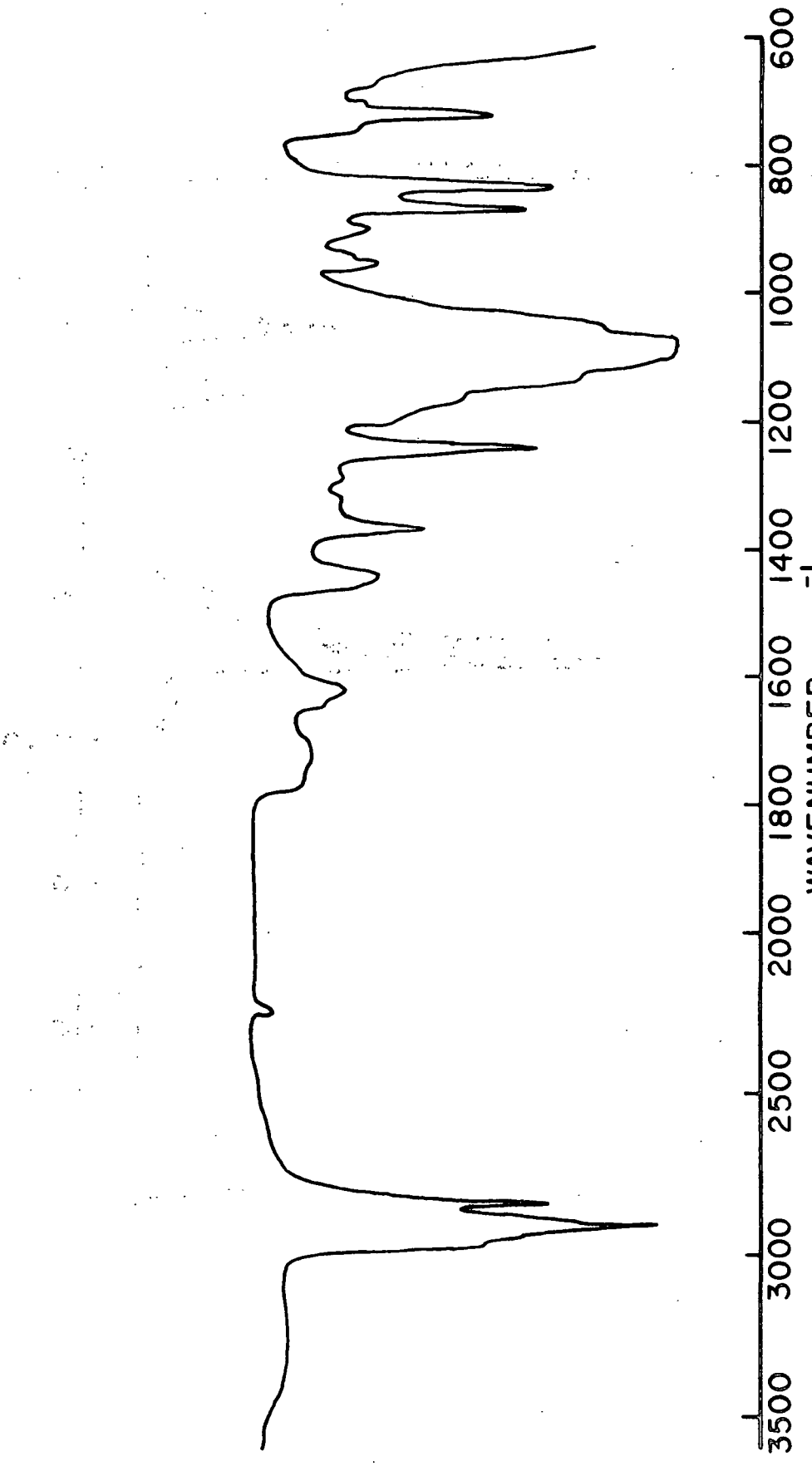


Figure 42. Infrared Spectrum of Unknown No. 1

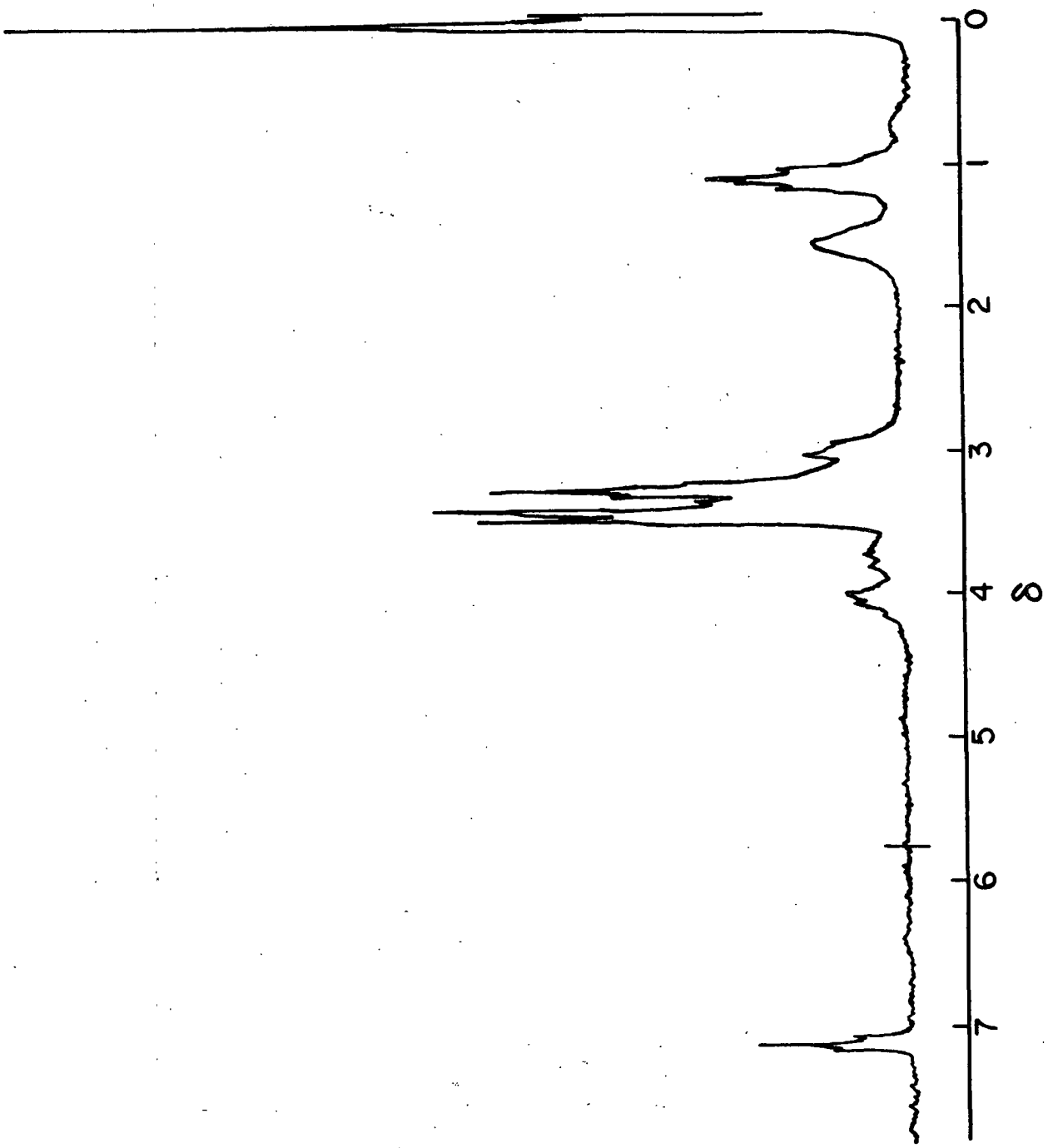


Figure 43. ^{1}H-NMR Spectrum of Unknown No. 1 in Chloroform-d Using Tetramethylsilane as Internal Reference

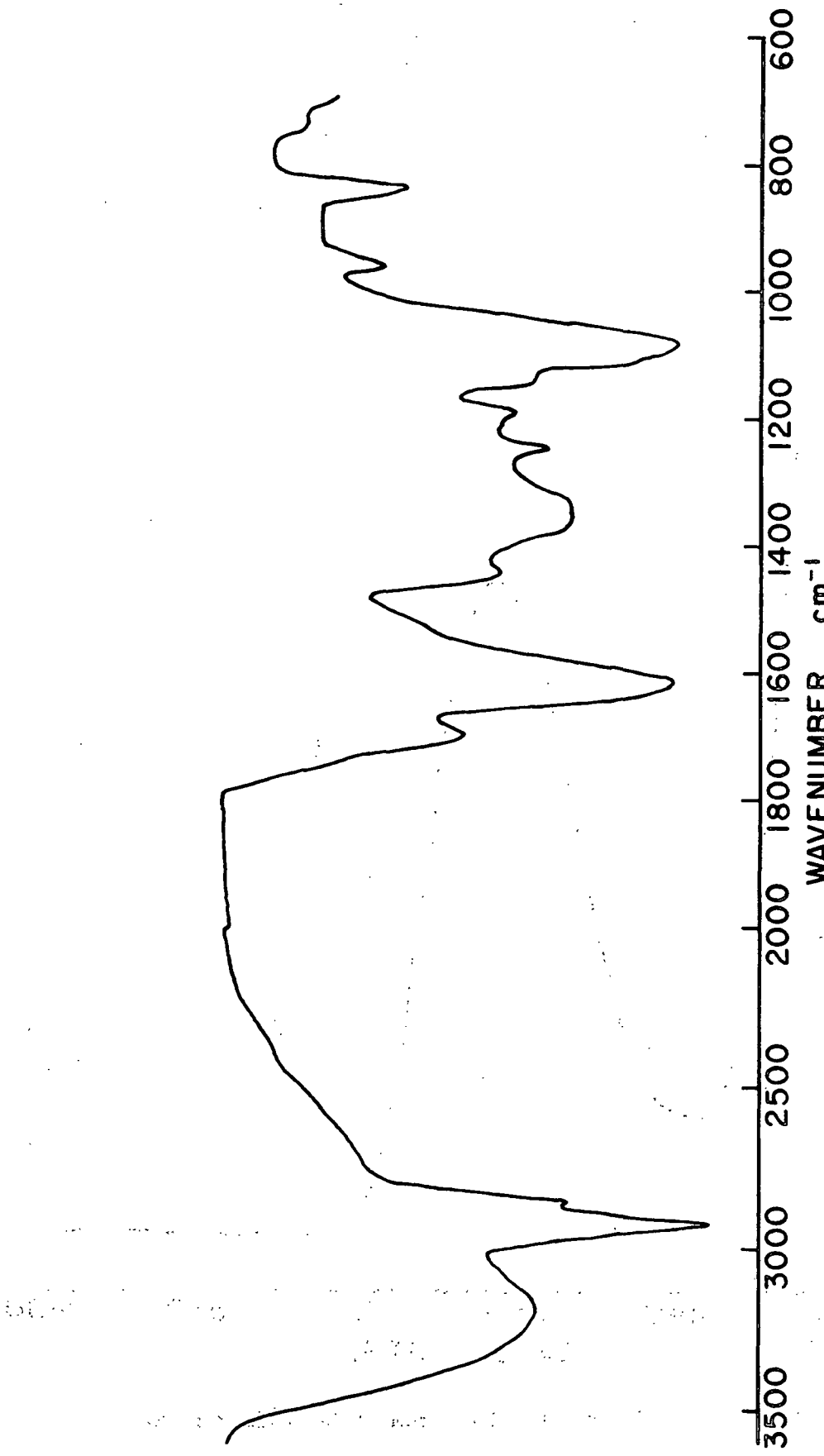


Figure 44. Infrared Spectrum of Unknown H

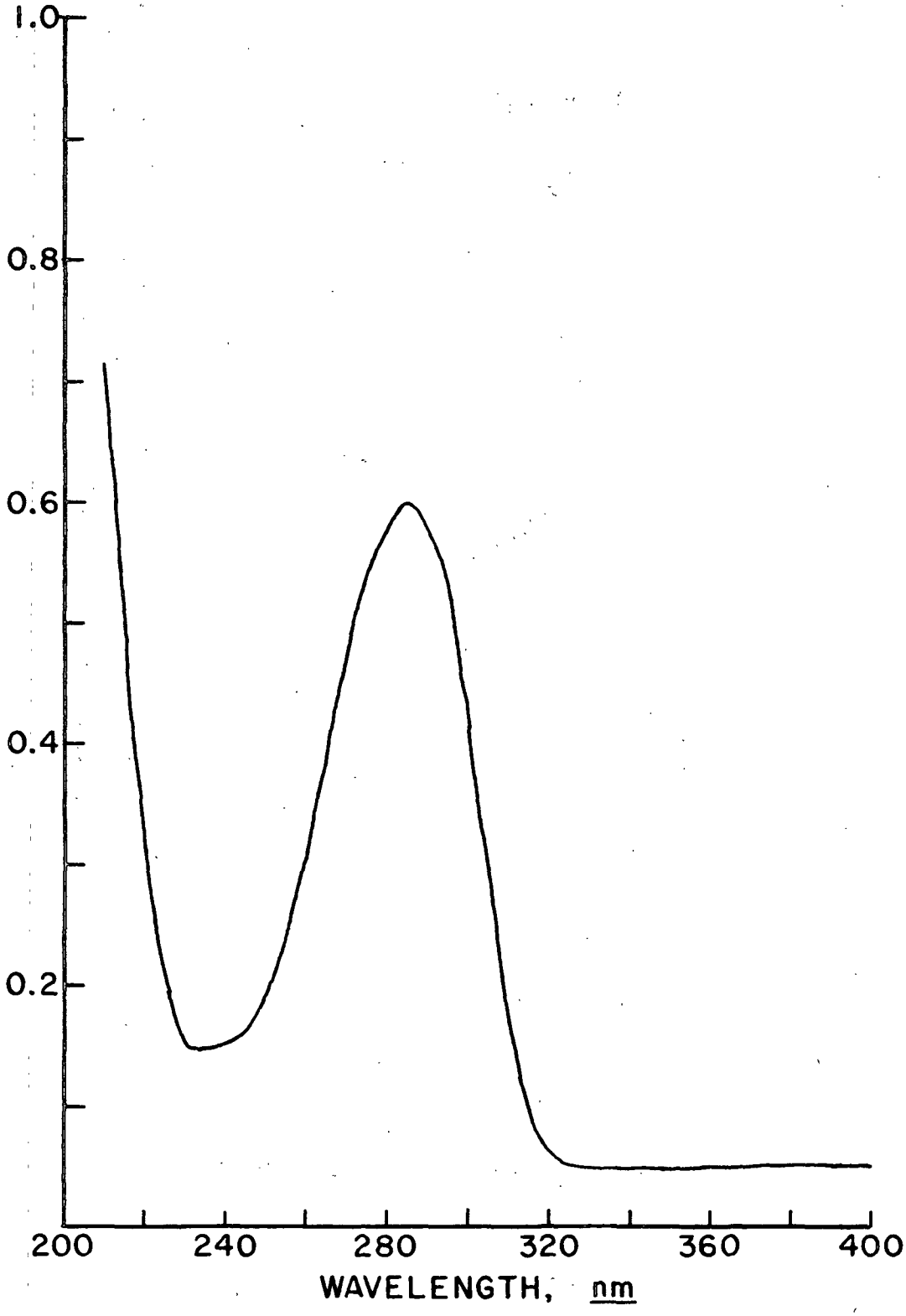


Figure 45. Ultraviolet Spectrum of Unknown H in Water